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ACAT2
gAcrp30/Adipolean
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Activin B
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ADAT1
Adiponectin
ADRP
AITRL
Akt1
Alpha-Feto Protein (AFP)
Alpha-Galactosidase A
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Angiopoietin-2 (Ang-2)
Angiostatin K1-3
Annexin-V
apo-SAA
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Apolipoprotein E2
Apolipoprotein E3
Apolipoprotein E4
APRIL
Artemin
ATF2
Aurora A
Aurora B
BAFF
BAFF Receptor
BCA-1 / BLC / CXCL13
BCMA
BD-1
BD-2
BD-3
BDNF
Betacellulin
Bivalirudin
BMP-2
BMP-4
BMP-6
BMP-7
BMP-13
sBMP-1A
Brain Natriuretic Protein
BRAC
Breast Tumor Antigen
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C5L2 Peptide
C-10
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C-Src
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Calbindin D-28K
Calbindin D-29K
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Calcitonin Acetate
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Cardiotrophin-1
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Caspase-6
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CD14
CD22
CD40 Ligand / TRAP

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CTGFL / WISP-2
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CXCL16
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E-selectin
ECGF
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Eotaxin-2
Eotaxin-3 (TSC)
EPHB2
EPHB4
Epigen
Epiregulin
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Erythropoietin (EPO)
Exodus-2
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Fas Receptor
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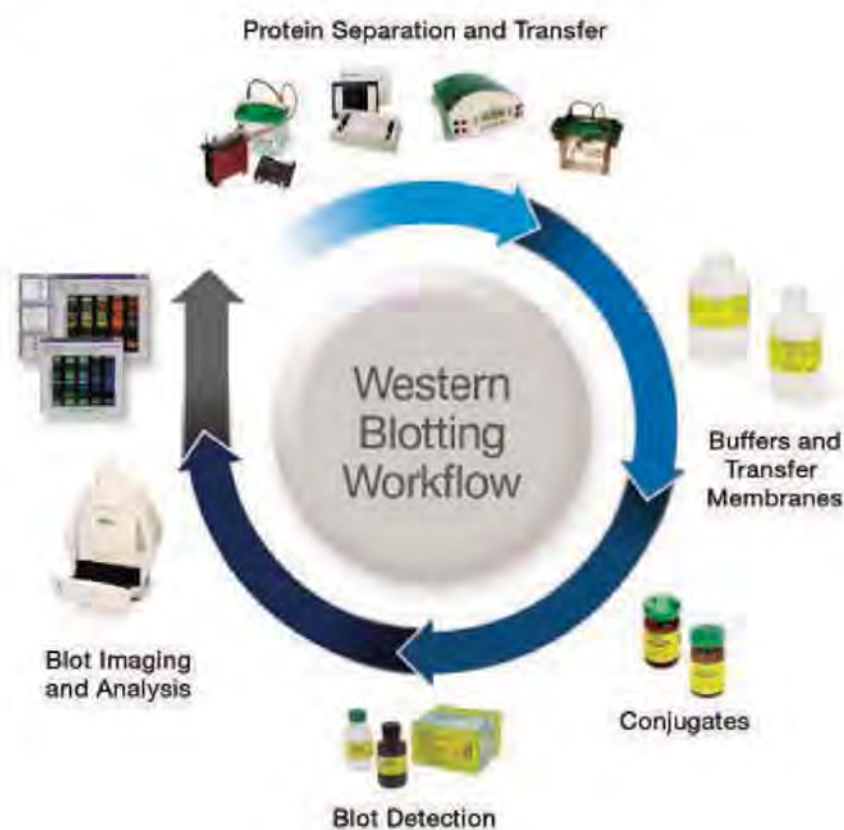
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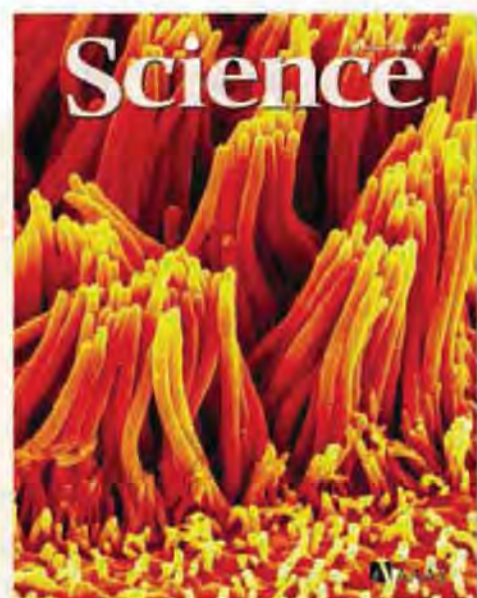
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COVER

Pseudocolored scanning electron micrograph (magnification ~34,000x) of the surface of mouse airway epithelia showing cilia protruding from epithelial cells; short protrusions in the foreground are microvilli from a nonciliated cell. In human airway epithelia, these motile cilia bear receptors that detect bitter compounds and signal the cilia to increase their rhythmic beat frequency to help clear noxious substances from the lungs. See page 1131.

Image: Tom Moninger (epithelia generated by Phil Karp)

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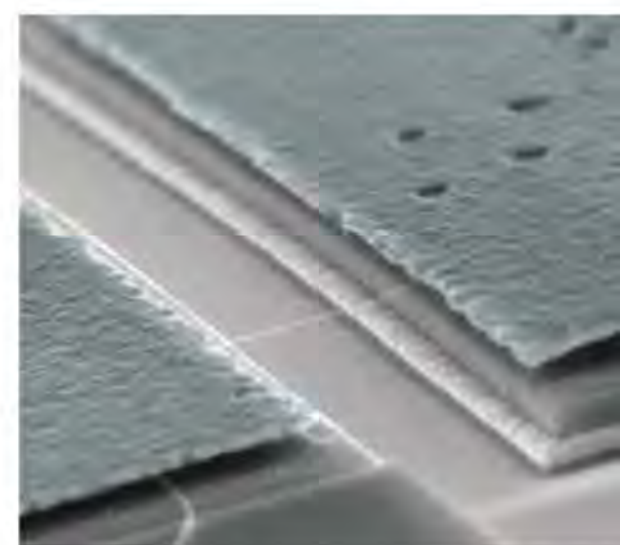
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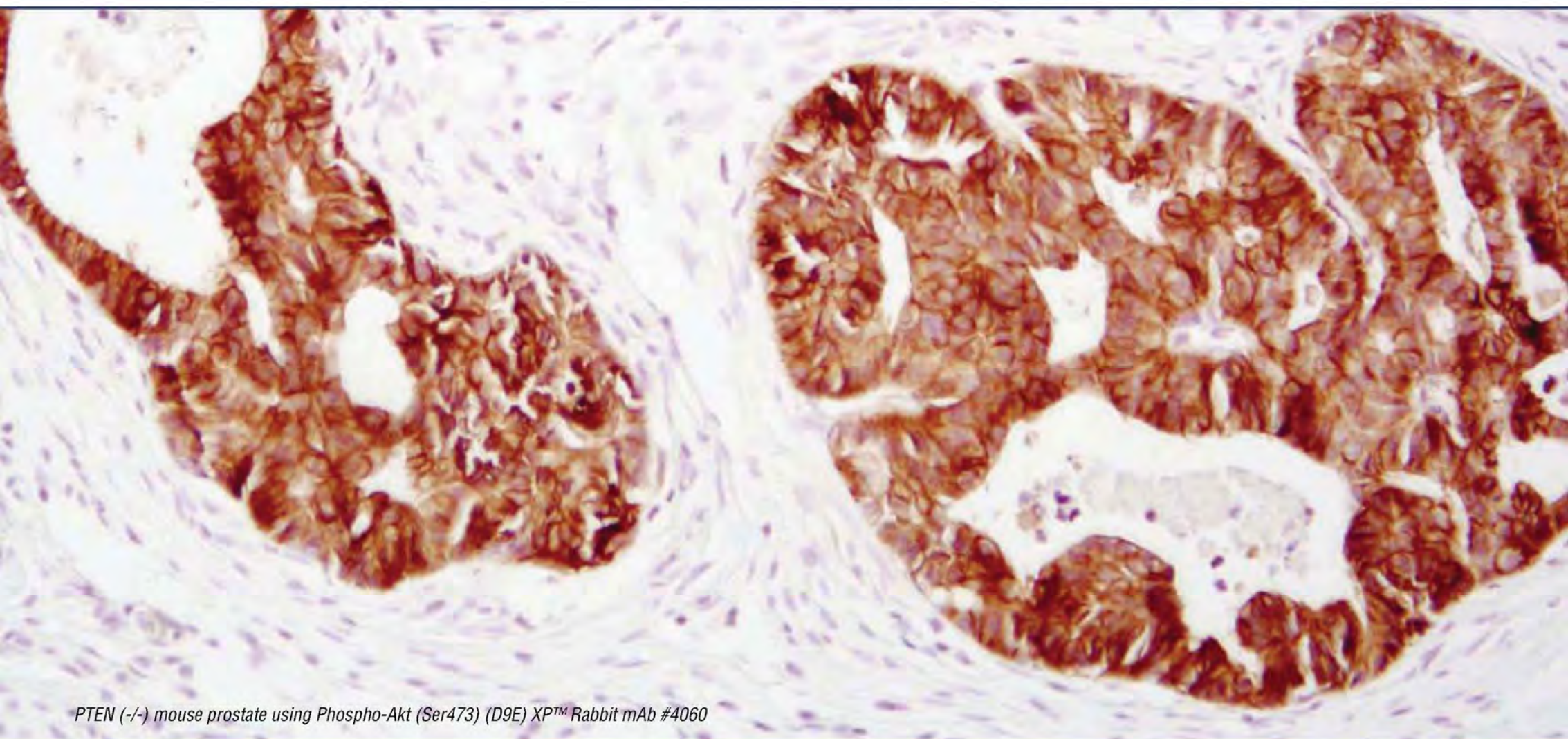
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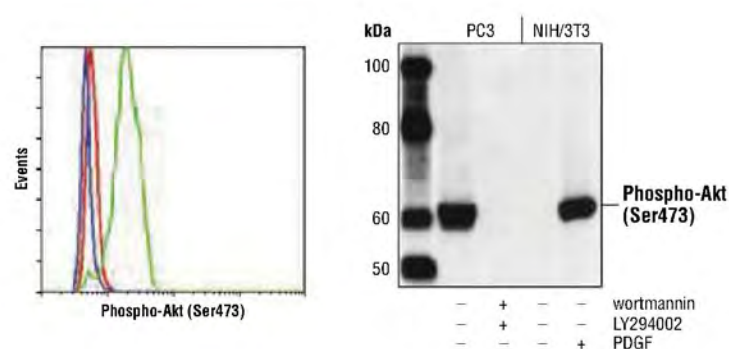
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Starvation Protects Germline Stem Cells and Extends Reproductive Longevity in *C. elegans*

G. Angelo and M. R. Van Gilst

During starvation, germline stem cells are saved for regeneration when food is restored.

10.1126/science.1178343

Coat Variation in the Domestic Dog Is Governed by Variants in Three Genes

E. Cadieu et al.

Huge variations in the coats of purebred dogs can be explained by the combinatorial effects of only three genes.

10.1126/science.1177808

Complete Resequencing of 40 Genomes Reveals Domestication Events and Genes in Silkworm (*Bombyx*)

Q. Xia et al.

Silkworm genomes show signatures of selection associated with domestication.

10.1126/science.1176620

>> *News story p. 1058*

Nitrous Oxide (N₂O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century

A. R. Ravishankara et al.

Nitrous oxide causes more stratospheric ozone destruction than any other ozone-depleting substance.

10.1126/science.1176985

>> *Science Podcast*

Chiral Organic Ion Pair Catalysts Assembled Through a Hydrogen-Bonding Network

D. Uraguchi et al.

A small cluster of hydrogen-bonded molecules acts as a highly selective asymmetric catalyst.

10.1126/science.1176758

TECHNICALCOMMENTS

Comment on "Floral Iridescence, Produced by Diffractive Optics, Acts as a Cue for Animal Pollinators"

N. I. Morehouse and R. L. Rutowski

full text at www.sciencemag.org/cgi/content/full/325/5944/1072-d

Response to Comment on "Floral Iridescence, Produced by Diffractive Optics, Acts as a Cue for Animal Pollinators"

H. M. Whitney et al.

full text at www.sciencemag.org/cgi/content/full/325/5944/1072-e

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RESEARCH ARTICLE: The VDAC2-BAK Rheostat Controls Thymocyte Survival

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The relative abundance of an anion channel and a proapoptotic protein determines thymocyte responses to death signals.

PERSPECTIVE: Controlling the Number of Tooth Rows

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Bmp4 distribution in the mouse jaw is restricted to prevent the induction of supernumerary teeth.

PERSPECTIVE: Epac2—A Molecular Target for Sulfonylurea-Induced Insulin Release

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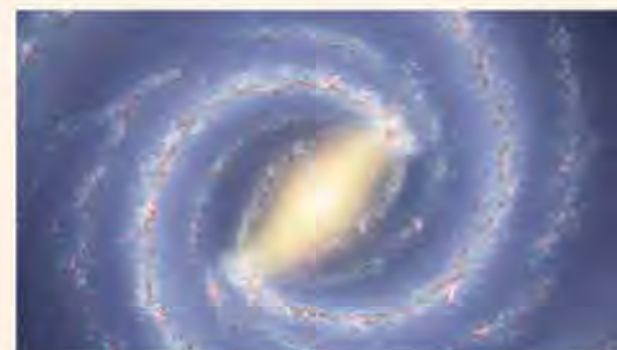
S. Gaidos

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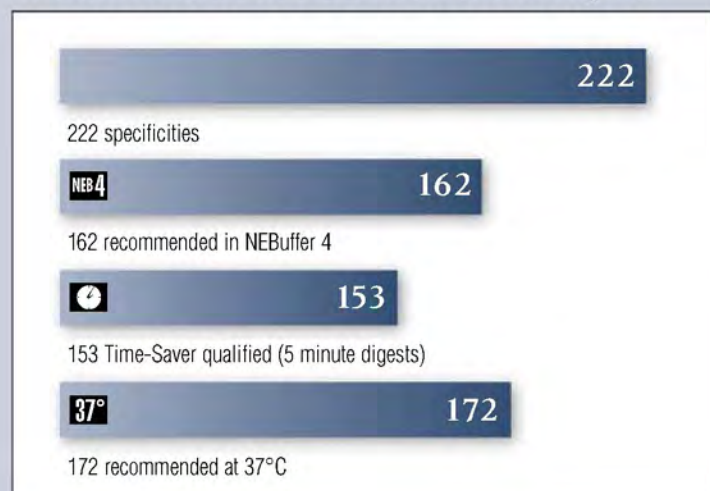
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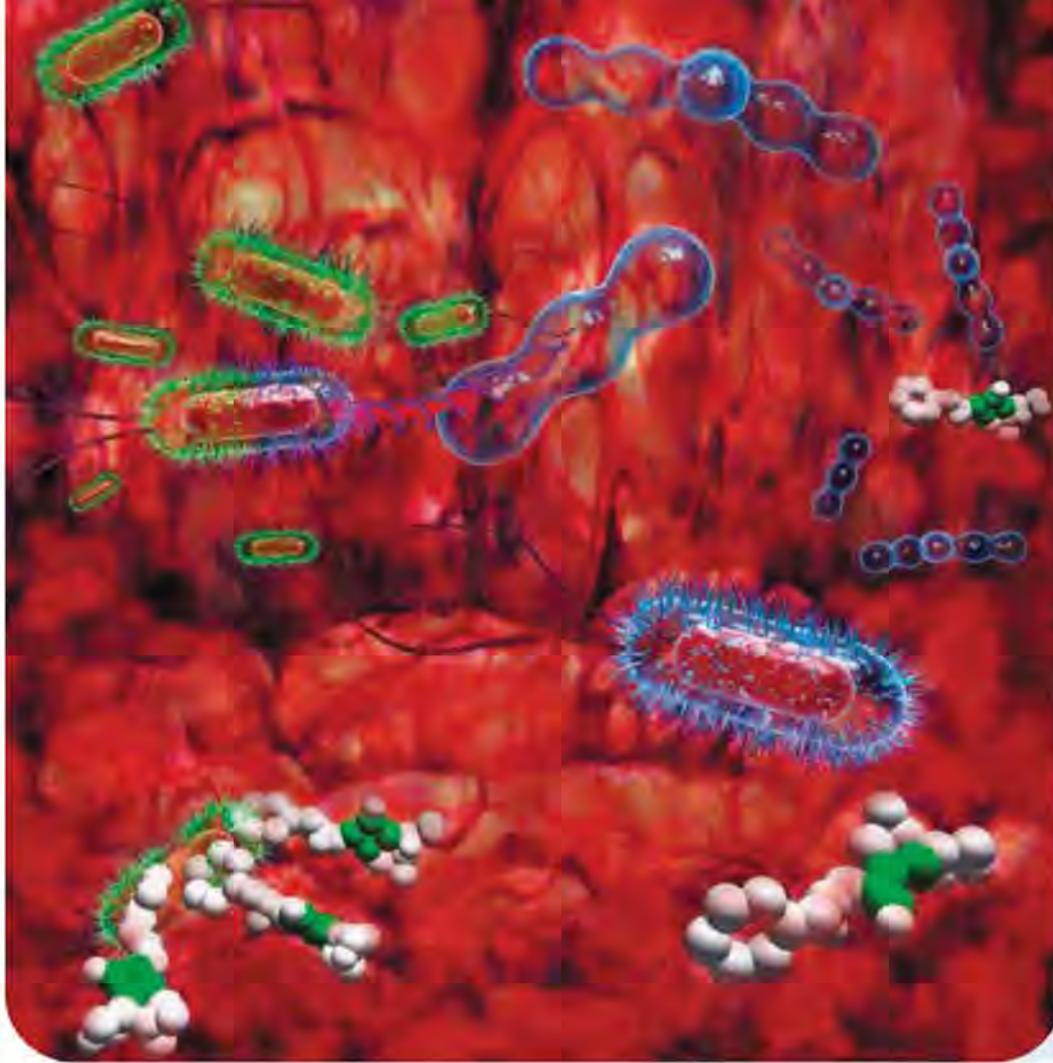
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<< Hidden Pockets of Resistance

Groups of bacteria indulge in gene swapping at frequencies correlated with prevailing selection pressures and phylogenetic relatedness. When assaulted by antibiotics, antibiotic resistance genes become favored currency for exchange among bacteria. During sequencing of human gut microflora, **Sommer *et al.*** (p. 1128) found a very large reservoir of distinct genes that, when put into *Escherichia coli*, conferred resistance to a wide range of drugs. By contrast, analysis of the culturable aerobic gut microbiome, which constitutes a tiny fraction of the entire gut flora, revealed resistance genes highly similar to those harbored by human pathogens. Although there is a risk of novel modes of antibiotic resistance emerging from this reservoir, because they are evolutionarily distant, gene transfer between pathogens and the poorly known majority of the microbiome might actually be quite restricted.

Toward New Scaffolds

Most existing antibiotics are derived from a small number of core molecular structures or scaffolds. As more and more pathogens emerge that are resistant to existing antibiotics, **Fischbach and Walsh** (p. 1089) review why renewed efforts must be made to find not only new antibiotics but new scaffolds. Approaches in the areas of natural products, synthesis, and target-based discovery are all yielding promising antibiotics candidates. The battle against resistance should also involve researching narrow-spectrum antibiotics and using combination therapies to extend the usefulness of drugs with high intrinsic resistance rates.

Adapting Coat Color

Simple phenotypic changes can often be the target of selection—for example, variations in coat color that provide protection against detection by predators. **Linnen *et al.*** (p. 1095) explore the underlying molecular mechanisms behind the production of pale deer mice living on the light-colored Nebraska Sand Hills. The mice that live on the sand are significantly lighter in color than conspecifics living nearby on darker soils. This lighter color was found to be due to *de novo* changes at the Agouti coat color locus. Thus, rapid adaptive change does not always rely on preexisting genetic variation.

Mind the Pseudogap

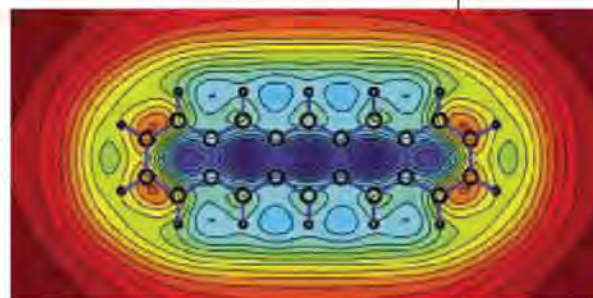
Below the transition temperature, an energy gap opens in superconductors, which effectively protects the superconducting phase. Above the transition temperature, the gap closes, creating exci-

tations and a loss of superconductivity. In the high-temperature superconducting cuprates, however, the gap persists above the transition temperature. Understanding the electronic structure of this pseudogap region is important in understanding the mechanism of superconductivity in the cuprates. **Lee *et al.*** (p. 1099; see the Perspective by **Norman**) use high-resolution, temperature-dependent scanning tunneling microscopy to reveal that the pseudogap regime is an incoherent (or phase-disordered) d-wave superconductor.

Atomic Imaging Within Adsorbed Molecules

Scanning tunneling microscopy provides atomic resolution images of surfaces and adsorbed atoms, but

imaging atoms within an organic molecule adsorbed on a surface is difficult because contrast is lacking in the states that determine the tunneling current. Atomic force microscopy should be able to resolve atoms through changes in short-range chemical forces, but resolution is lost if the scanning tip undergoes modifications or if it moves the molecule. **Gross *et al.*** (p. 1110) show that in situ functionalization of the tip—for example, with CO—can dramatically improve the resolution of images of pentacene molecules adsorbed on conducting surfaces, like copper, and nonconductors, like NaCl.



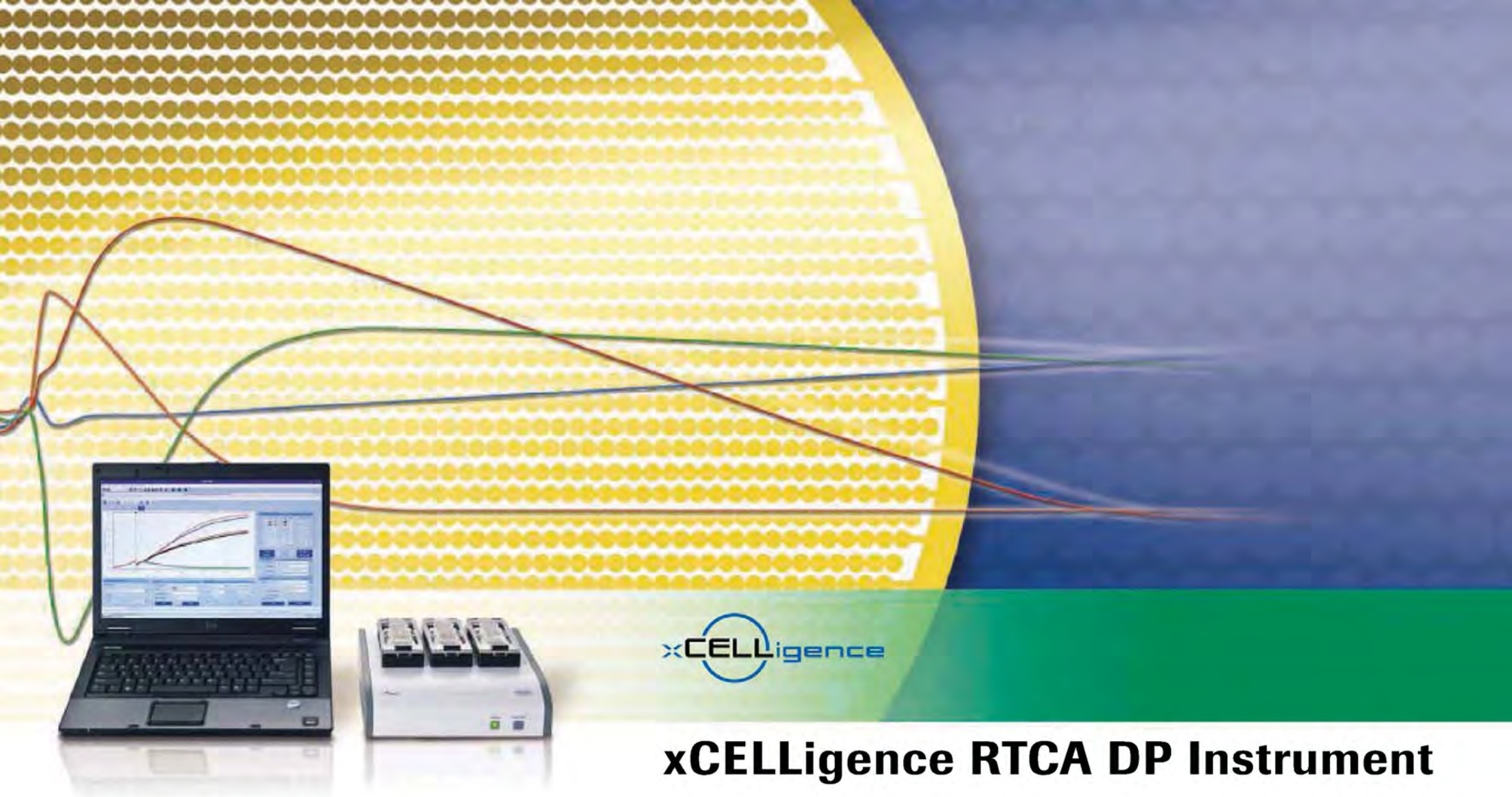
More Than the Sum of the Parts

The radiative output of the Sun varies distinctly with the 11-year cycle of sunspots, although the change in energy output is small—less than a tenth of a percent in magnitude. Nevertheless, that small variation produces changes in sea surface temperatures two or three times as large as it should, and the mechanism by which this occurs has remained unclear. **Meehl *et al.*** (p. 1114; see the news story by **Kerr**) employ three global, coupled climate models to simulate this phenomenon. Two mechanisms appear to act in conjunction to cause this ocean response: a change in the abundance of stratospheric ozone owing to fluctuations of shortwave solar forcing; and a coupled surface ocean-atmosphere response. This combination of effects enhances precipitation maxima, reduces low-latitude cloud cover, and lowers the temperature of surface waters in the tropical Pacific Ocean, resulting in the larger warm-to-cold variation.

“Rapid” Recovery

The Permian-Triassic extinction 252 million years ago was Earth's most severe biotic crisis since the Precambrian and is thought to have depressed diversity in its wake for millions of years. **Brayard *et al.*** (p. 1118; see the Perspective by **Marshall and Jacobs**) show, however, that ammonoids, a large group of marine organisms that were severely affected, recovered remarkably quickly. Only 1 million years after the extinction, ammonoids had recovered to levels higher than in the Permian,

Continued on page 1045



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Continued from page 1043

compared with the 10-million-year biotic recovery period for other benthic organisms. The Triassic recovery seems to include several cycles, but the immediate recovery of ammonoids may have left them as one of the most diverse groups in the earliest Triassic.

Restoring Oysters

Populations and wild fisheries of native oyster species have collapsed worldwide because of overfishing and habitat destruction, resulting in severe ecosystem alteration and degradation. Expensive restoration efforts have met with little success, leading to the introduction of non-native oyster species in an attempt to recover lost economic and ecological benefits. In the Chesapeake Bay on the U.S. East Coast, eastern oyster landings and population abundance have plummeted to approximately 1% of historical levels, despite considerable expensive attempts to restore the fishery. **Schulte *et al.*** (p. 1124, published online 30 July) present field evidence of a successful restoration of a large metapopulation of native oysters in the Great Wicomico River, a tributary on the western shore of the Chesapeake Bay. The metapopulation is composed of a network of reef complexes spanning 35 hectares and comprises an estimated 185 million live native oysters of 1-year-old juveniles and 2- and 3-year-old adults.

T_{reg} Responses to Eos

CD4⁺ regulatory T cells (T_{regs}) are critical for keeping our immune system in check: They prevent immune responses from getting out of hand and keep autoimmunity at bay. By activating the expression of some genes and turning off expression of others, the master regulatory transcription factor of T_{regs}, Foxp3, endows these cells with the appropriate gene expression program to mediate their suppressive effects. **Pan *et al.*** (p. 1142, published online 20 August) now demonstrate that the transcription factor Eos is selectively required for Foxp3-mediated gene suppression in mice. Genes normally suppressed by Foxp3 in T_{regs} remained "on" when Eos expression was suppressed, whereas genes activated by Foxp3 were unaffected. T_{reg} function was also affected by Eos suppression. With half their genetic program disrupted, these cells resembled an intermediate between T_{regs} and conventional CD4⁺ T cells—unable to suppress immune responses properly and partially responsive to T cell-activating stimulation.



Beat It

Primary cilia are specialized organelles that serve important sensory functions in many different tissues and cells, and defects in their structure and function underlie a variety of genetic diseases. In contrast to primary cilia, motile cilia serve a mechanical function. For example, the cilia on airway epithelia remove inhaled material from the lung. **Shah *et al.*** (p. 1131, published online 23 July; see the cover; see the Perspective by **Kinnamon and Reynolds**) now show that these classic motile cilia are also chemosensory. The motile cilia on airway epithelia contain bitter-taste receptors and their associated signaling machinery. Moreover, application of bitter substances triggers an elevation of intracellular Ca²⁺ levels and increases cilia beat frequency. Thus, in airway epithelia, bitter-taste receptors may be able to detect noxious substances entering the airways and initiate an autonomous defensive mechanism designed to accelerate elimination of the offending compound.

Tapping the Mitochondrial Proteome

Mitochondria produce the energy that cells need to survive, function, and divide. A growing list of human disorders has been traced to defects in mitochondrial function. About 300 mammalian mitochondrial proteins are functionally uncharacterized, and **Hao *et al.*** (p. 1139, published online 23 July) reasoned that the most highly conserved proteins within this group might provide insights into human disease. A combination of bioinformatics, yeast genetics, biochemistry, and human genetics was used to show that a previously uncharacterized mitochondrial protein (Sdh5) is required for the activity of respiratory complex II. Inactivating mutations in the human gene encoding *SDH5* were found in individuals with hereditary paraganglioma, a rare neuroendocrine tumor. Thus, analysis of a mitochondrial protein in yeast has revealed a human tumor susceptibility gene.

CREDIT: PAN ET AL.

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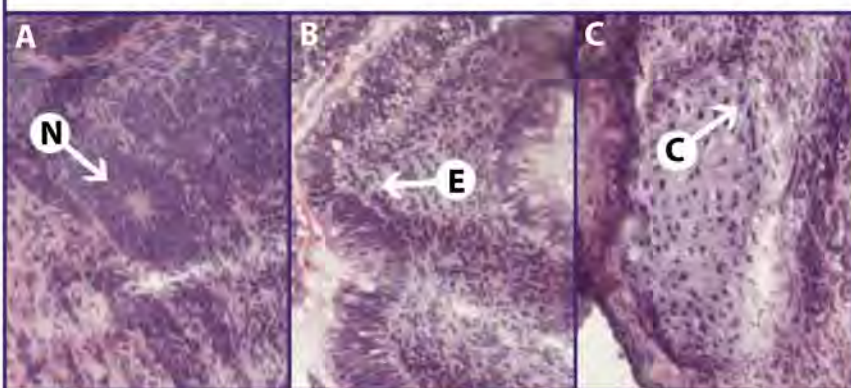
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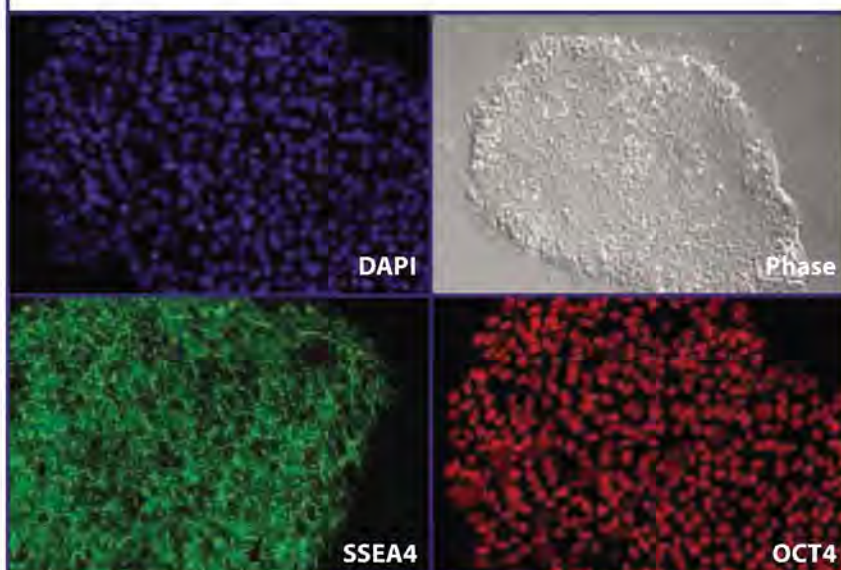
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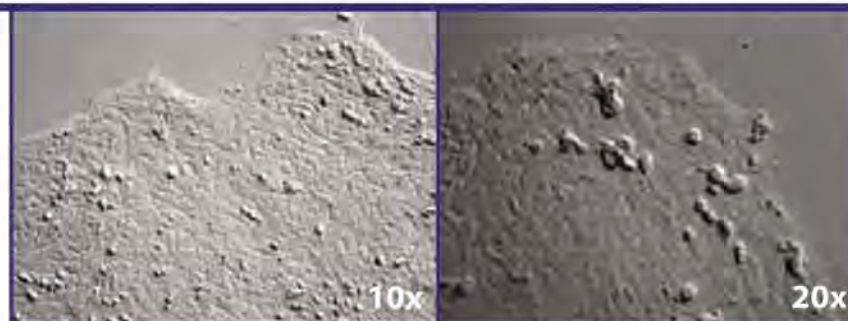
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Maxine Singer is president emerita of the Carnegie Institution of Washington in Washington, DC.

Great Teachers for STEM

MANY COUNTRIES ARE STRIVING TO DEVELOP THE HUMAN RESOURCES REQUIRED TO ADVANCE science and technology. Success requires a system of education that prepares young people for life in today's complex societies. The Obama administration has recently stressed the need to strengthen science, technology, engineering, and mathematics (STEM) education in the United States. U.S. Secretary of Education Arne Duncan clearly understands that the quality of STEM teaching is of singular importance to the success of students, requiring "great teachers, who know the content."* Herein lies a major challenge: How to develop and cultivate great STEM teachers?

U.S. schools currently fail to teach STEM effectively. Evidence includes the low standing of U.S. students in international comparisons; for example, in the 2006 Program for International Student Assessment (PISA, conducted by the Organization for Economic Cooperation and Development), American 15-year-olds ranked 24th out of 57 countries in science and 32nd in mathematics. Other distressing indicators are that only 40% of Americans accept the fact of biological evolution, and less than half of American adults can provide a minimal definition of DNA.† Thanks to the First Amendment to the U.S. Constitution, creationism and intelligent design may not be taught in U.S. public schools. But evolution is not generally taught adequately, even when included in school curricula.

Some scientists, nationwide, have for decades provided professional development in content and pedagogy to teachers of math and science, from early through secondary (precollege) education. But test data and experience indicate that, with some remarkable exceptions, even intensive professional development is a failed experiment that cannot make up for the poor quality of the teachers' own STEM educations. Many elementary school teachers studied no science or math beyond high school and may remember only that they disliked the subjects. Secondary school STEM teachers who were educated decades ago are unlikely to be familiar with modern scientific knowledge. Most troubling, many secondary school classes are taught by teachers with no STEM qualifications at all. The U.S. scientific community has largely ignored the problem of ill-prepared STEM teachers, except perhaps when troubled about their own children's school experience. Now this community must energetically engage this challenge by spearheading several initiatives.

Excellent undergraduate students who are not dedicated to research should be encouraged by their science faculty to become STEM teachers. Graduate students in a research field generally receive tuition grants and stipends; those who pursue advanced degrees and certification for STEM teaching should be offered fellowships as well. The U.S. National Science Foundation's new Teaching Fellowships program should be expanded so that it can more widely provide funds to support tuition, stipends, and academic programs for those with undergraduate degrees in STEM subjects who commit to teaching in high-need school districts. This program can attract people with STEM skills who are now jobless into teaching careers.

The science community needs to participate directly in improving teacher education programs. Scientists should work to help develop STEM teacher education programs that are rigorous and relevant for teaching students who have grown up in the Internet era. Successful models, such as UTeach at the University of Texas, Austin, are already being replicated at 13 other universities across the United States through the National Math and Science Initiative (www.nationalmathandscience.org). But this type of program should be expanded with federal funds, as recommended in the landmark *Rising Above the Gathering Storm* report of 2005 from the U.S. National Academies.

Last but not least, STEM professionals must engage actively with precollege-level STEM teachers in a sustained way. In his memoirs, physicist and Nobel Peace Prize recipient Andrei Sakharov describes his father, a high-school physics teacher, as a physicist. STEM teachers must similarly be considered vital members of the professional scientific community.

— Maxine Singer

10.1126/science.1176999

*J. Mervis, *Science* **324**, 159 (2009). †J. D. Miller, E. C. Scott, S. Okamoto, *Science* **313**, 765 (2006).



GEOLOGY

Not of a Certain Age

At present, there are 174 characterized impact structures on Earth, ranging from the largest—the 2-billion-year-old Vredefort that stretches 300 km across South Africa—to about 20 or so structures smaller than the 1-km-diameter Barringer crater in Arizona; most of these are less than 1 million years old. Although these structures represent only the preserved fraction of the overall series of asteroid impacts on Earth, their age-size relationships might aid in assessing the impact flux or the relation of impacts to events such as extinctions (for example, that marking the Cretaceous-Paleogene boundary). As pointed out by Jourdan *et al.*, however, many of the craters are in fact poorly dated. About half have only approximate ages based on stratigraphy. Moreover, although 25 of the craters have ages reported with uncertainties of less than 2%, many of these reports, on investigation, contain generous or misleading estimates; in the authors' assessment, only 11 of the 25 craters have been assigned accurate ages. Thus, it is difficult to infer much from the current crater data about periodicities or changes in impacts through time. — BH

Earth Planet. Sci. Lett. 10.1016/j.epsl.2009.07.009 (2009).

GEOCHEMISTRY

Heavy Down Below

Many chemical and biological processes skew, or fractionate, atomic isotope ratios in products relative to their initial relative abundances. For example, most of the iron in Earth's crust exists as ^{56}Fe , but iron that undergoes reduction/oxidation (redox) reactions is on average slightly lighter (that is, enriched in ^{54}Fe —even by just a few parts per thousand).

To determine the extent of contemporary Fe redox cycling in different marine settings, Homoky *et al.* compared Fe isotope signatures from pore water collected on a shallow continental shelf to those from pore water associated with sediments as deep as 4222 m below sea level, at the bottom of the Southern Ocean. The significant fractionation of ^{56}Fe isotopes in shallow sediments indicated extensive Fe reduction by active communities of bacteria; in contrast, the Fe isotopes in the deep sediments showed almost no fractionation, thereby suggesting that deep marine sediments undergo very little redox cycling. Although limiting organic matter is expected to restrict the activity of

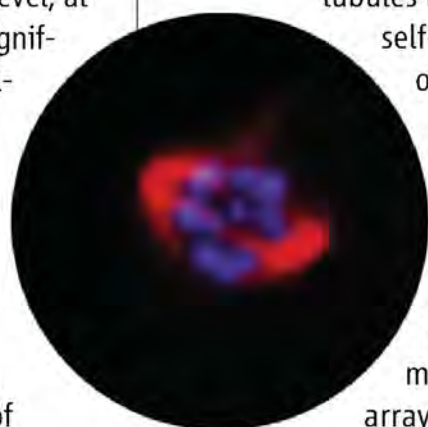
iron-reducing bacteria at depth, the degree of fractionation is also dependent upon the reactivity of Fe-oxide minerals. Defining the controls on stable isotope fractionation in sediments will not only aid in understanding modern biogeochemical processes, but will also allow for a more accurate interpretation of Earth's history based on isotopic signatures found in the rock record. — NW

Geology 37, 751 (2009).

CELL BIOLOGY

Size Matters

The mitotic spindle, which pulls apart the duplicated chromosomes during cell division in animal and plant cells, is composed of microtubules that have been found to self-assemble in the presence of chromosomes. Dinarina *et al.* have developed a cell-free system in order to monitor the effects of chromosome size and shape on spindle morphology. Microscopic chromatin-coated beads were arrayed on a glass slide to which



cytoplasmic extracts from *Xenopus laevis* were applied, which made it feasible to look at many individual spindles assembling simultaneously. When chromatin (blue) was deposited as circular spots 15 μm in diameter, typical bipolar spindles (red) were observed, and larger spots tended to produce spindles with additional poles. In contrast, for chromatin patterned in a chromosome-like rectangle with dimensions of 6 x 18 μm , a typical bipolar spindle orthogonal to the long axis of the chromosome was seen. Longer chromatin arrangements generated multipolar, disorganized spindles, which also generally crossed the long axis of the chromosome, and thicker arrangements generated multiple half-spindles along one side of the patch of chromatin. Thus, the size and shape of chromosomes can define microtubule spindle shape and orientation. — SMH

Cell 138, 502 (2009).

PLANT SCIENCE

Adding Oregano to Corn

Many plants emit signals to augment their defenses against attack by insects; some of these signals are known to work by attracting

CREDITS (TOP TO BOTTOM): JUPITERIMAGES; DINARINA ET AL., CELL 138, 502 (2009)

predators of the attackers. Maize roots under threat from larvae of the western corn rootworm normally emit the terpene caryophyllene, which serves to mobilize nematodes that then kill these larvae. However, most cultivated maize has lost the capacity to produce this terpene.

Degenhardt *et al.* have engineered transgenic maize plants that carry a caryophyllene synthase gene from oregano. This manipulation restored the production of this compound, with the consequence that nematodes reduced the number of rootworms by more than half, resulting in much less root damage. This finding confirms that nematodes can be recruited effectively by caryophyllene and provides the basis for a pest biocontrol strategy to improve cultivated plants. — LMZ

Proc. Natl. Acad. Sci. U.S.A. **106**, 13213 (2009).

BIOMEDICINE

Blood Relations

The mortality rate for pancreatic cancer approaches the rate of incidence, in part because early stages of the disease are asymptomatic and diagnosis at later stages remains difficult. As with many cancers, a family history of



Genetic associations with pancreatic cancer (sorted by chromosome).

the disease is a known risk factor; identification of genetic variants associated with pancreatic cancer might improve early detection.

Amundadottir *et al.* have performed a genome-wide association study to identify common genetic variants found specifically in patients suffering from pancreatic cancer. In a Manhattan plot, they found within the first intron of the *ABO* blood group gene variants that were associated with susceptibility to the disease. The *ABO* gene encodes a glycosyltransferase that modifies a cell surface antigen. The three alleles (A, B, and O) of this gene determine blood type, which was first associated with gastric and pancreatic cancer more than half a century ago. What pathways might mediate this association? Variants within the same intron of the *ABO* gene have also been linked with circulating levels of the cytokine tumor necrosis factor α , which is

involved in the inflammatory response. Other possible factors contributing to tumorigenesis include alterations in cell adhesion and immune surveillance. — HP

Nat. Genet. **41**, 10.1038/ng.429 (2009).

BIOMATERIALS

Delivering to the Senses

Though nerve cells can be stimulated externally by electrical impulses, controlled delivery of molecular neurotransmitters would enhance the selectivity of the activation process across cell types. Simon *et al.* used conducting organic polymers to create an encapsulated device for delivering neurotransmitters such as glutamate in either continuous or pulsed fashion under electronic control. The device was initially constructed in a planar geometry for in vitro testing, which achieved glutamate-induced spikes in intracellular Ca^{2+} concentration in astrocytes. For in vivo testing, the device was reconfigured into a syringe-like geometry in order to deliver glutamate to the cochlea of anaesthetized guinea pigs, thereby triggering a variation in auditory brainstem response. Based on the current configurations, the devices could be operated in either continuous delivery mode for up to 1 hour or in pulsed delivery mode for much longer periods of time. — MSL

Nat. Mater. **8**, 10.1038/nmat2494 (2009).

IMMUNOLOGY

Paracrine Pass-Through

Upon encounter with infectious agents such as viruses, host organisms must rapidly mount a strong system-wide immune response, lest the infection spread dangerously wide. How do they accomplish this task at the early stage when so few cells are typically infected? Patel *et al.* demonstrate that one mechanism may rely on intercellular communication through gap junctions. They showed that when cells were exposed to double-stranded DNA (dsDNA), which can be found during infection by some types of viruses, not only the cell that directly sensed the dsDNA, but also the surrounding cells, were able to produce pro-inflammatory and antiviral cytokines that are required to initiate the full antiviral immune response. If the gap junctions were blocked through chemical inhibition or by genetic interference, the surrounding cells no longer produced cytokines when their neighbors were exposed to dsDNA. Thus, gap junctions allow the rapid mobilization and amplification of an antiviral immune response by transmitting the activating signal directly from infected cells to uninfected cells. — KLM

Proc. Natl. Acad. Sci. U.S.A. **106**, 12867 (2009).

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It's Fit to Be Smart

Brainy bowerbirds get more dates than sluggish problem-solvers, say behavioral ecologists at the University of Maryland, College Park.

Australian bowerbirds exhibit complex mating behavior that involves singing, dancing, and building cozy twig-and-grass bowers to lure females. To see if male IQ contributes to reproductive fitness, graduate student Jason Keagy led two experiments at Wallaby Creek in Australia that were based on males' dislike of having red things in their bowers. In one test,

birds had to drag off a transparent box covering a red plastic battery cover. In another, birds tried to hide immovable red tiles from view.

Among about 30 birds, the speediest problem-solvers also achieved the most sexual encounters, the researchers report in a paper in press in *Animal Behaviour*. "This is the first test of general cognitive ability [or 'g'] being related to mating success for any species," Keagy says. "This is a really nice observation," says Carlos Botero, a behavioral ecologist at the National Evolutionary Synthesis Center in Durham, North Carolina. But, he adds, "at this point, we don't know" if females are choosing mates on the basis of brains or on something such as a sexy dance that may not correlate with *g*.

Multitasking—Bad for The Brain?

Scientists at Stanford University in Palo Alto, California, have unsettling news from what they say is the first-ever study of chronic multitaskers.

A team headed by psychologist Eyal Ophir compared 19 "heavy media multitaskers" (HMMs), identified by questionnaires on media

use, with 22 "light media multitaskers" (LMMs). They tested how well the subjects could filter relevant information from the environment, filter relevant information in their memories, and quickly switch cognitive tasks. One filtering test, for example, required viewers to note changes in red rectangles while ignoring blue rectangles in the same pictures.

HMMs did worse than LMMs across the board. Surprisingly, says co-author Clifford Nass, "they're bad at every cognitive control task necessary for multitasking." Nass, a sociologist, says the study has "disturbing" implications in an age when more and more people are simultaneously working on computers, listening to music, surfing the Web, and texting or talking on a phone. Also troubling, he notes, is that "people who chronically multitask believe they're good at it." The findings are reported this week in the *Proceedings of the National Academy of Sciences*.

The team hopes to investigate whether multitasking really scrambles brains or whether people with poor filtering and attentional abilities are more attracted to it to begin with.

Psychologist Anthony Wagner suspects that media multitasking offers instant rewards that reinforce "exploratory" behavior at the expense of the ability to concentrate on a particular task.

Scopes in Oils

Galileo Galilei has been credited for making telescopes popular, but a fresh analysis of masterpieces by Jan Brueghel the Elder shows that telescope technology spread more quickly than historians realized. Pierluigi Selvelli and Paolo Molaro, astronomers at the Osservatorio Astronomico di Trieste in Italy, recently observed that a painting called *Extensive Landscape with View of the Castle of Mariemont* portrays what was very likely one of the first telescopes ever built. The painting, showing a 40-centimeter-long Dutch spyglass, is dated to about 1608–11. Telescopes first appeared in 1608.

Brueghel also tucked a 1.8-meter collapsible telescope into *Allegory of Sight*, painted with Peter Paul Rubens in 1617. Johannes Kepler described the design in 1611 but never built one. The painting, the scientists report in a 20 August paper on the arXiv preprint server, shows the sophisticated instrument was in use earlier than anyone thought.

Brueghel's *Extensive Landscape with View of the Castle of Mariemont* (detail).



When Zombies Arrive

If zombies attack, the living must show no mercy. So concludes a mathematical model that its creator says could cast light on outbreaks of infectious disease.

The virtual scenario, designed by epidemiologist Robert Smith? (*sic*) of the University of Ottawa in Canada, models an outbreak of slow-moving, flesh-eating "living dead" who reproduce by biting the living and can be killed only by a blow or a gunshot to the head. The model includes the resurrection and zombification of the dead as well as the effects of quarantining the infected and possibly rehumanizing them. "We tried to describe as much of the zombie biology as we could," says Smith?, in a new book titled *Infectious Disease Modelling Research Progress*.

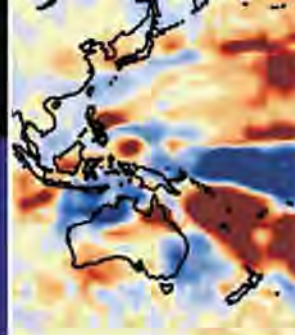
The only way to get rid of the undead for good is to launch frequent and large-scale attacks and "destroy their brains," notes Smith?. Mike Tildesley, an epidemiologist at the University of Edinburgh in the United Kingdom, sees an "interesting analogy" with infectious diseases: Whatever the control strategy—vaccination, quarantine, or eradication—it's important to stick to the plan."





Silkworm domestication

1058



Solar variability and climate

1058

HUMAN GENETICS

Cash-Starved deCODE Is Looking For a Rescuer for Its Biobank

Human geneticists are keeping a close eye on the health of Iceland's deCODE Genetics. The genomics company announced earlier this month that it has enough cash to keep going for only a few more weeks. If it halts its research efforts, that would bring to an end a remarkably productive run in the search for genes underlying common diseases. The company's troubles are also raising questions about the future of its massive DNA biobank, which contains a wealth of genetic data on the Icelandic population.

deCODE says it is talking to potential academic as well as commercial partners as part of a restructuring. The Wellcome Trust, the giant U.K. biomedical research charity, is said

p. 13). That plan was later shot down by Iceland's courts for infringing on privacy. In the meantime, the company began building a DNA, genealogical, and medical database by recruiting volunteers—a conventional approach that “has never caused much tension,” says Jon Johannes Jonsson, a medical geneticist at the University of Iceland in Reykjavík who opposed deCODE's earlier proposal. The database now covers about 140,000 people, says deCODE. Geneticists say its combination of large size and detailed genealogical data is probably unique.

The biobank has been a boon for genome-wide association studies (GWAS), which scan the entire genome for disease risk markers.

deCODE announced that it had only \$3.8 million in cash left and “sufficient resources to fund operations only into the latter half of the third quarter.” It plans to sell its medicinal chemistry, structural biology, and drug programs and focus on genetic tests.

deCODE spokesperson Edward Farmer says the company is “talking to a whole range of present and potential customers and partners from pharma to biotech to government and academic groups.” Included are “just about all of the big names in human genetics,” Farmer told *Science* by e-mail.

Some observers hope that a rescue by Wellcome, if it happens, would broaden access to deCODE's data. Although the company has collaborated with many outside groups, it has turned some down, and it does not make its data sets publicly available the way GWAS researchers now funded by Wellcome do.

Adding to the allure, deCODE's data “will become even more valuable,” predicts Jonsson. Gene hunters are now searching for rare risk variants by “deep sequencing” specific regions of DNA, an approach that requires studying families—which deCODE's biobank has in abundance.

A wholesale transfer of the biobank to the United Kingdom is not a sure thing, however. Icelanders' genetic data could be moved to another European Union country, but all personal identifiers would have to be removed or made anonymous, says Sigrún Jóhannesdóttir, director of the Icelandic Data Protection Authority. Another problem would be the use of biological samples and clinical data, which were collected by clinicians at Icelandic hospitals and institutes. Collaborative agreements that are part of deCODE's legal filings—as well as informed consent documents signed by the volunteers—generally state that DNA samples and medical data must be returned to the doctors when deCODE's studies are completed, notes Vilmundur Gudnason, director of the Icelandic Heart Association.

This means that any transfer would require agreements from deCODE's clinical partners and, potentially, modified informed consent agreements. One possible way to avoid such obstacles, some suggest, would be for the company to spin off an academic organization in Iceland with Wellcome Trust support. With deCODE's cash diminishing fast, time is running out to reach an agreement.

—JOCELYN KAISER



Dismantling. deCODE is selling much of its work to focus on genetic testing.



to be in discussions with the company to take over support of the biobank. (Wellcome declined to comment.) But Iceland's data privacy laws and deCODE's agreements with clinicians could preclude transfer of the biobank outside the country.

deCODE and its CEO, geneticist Kári Stefánsson, drew controversy 11 years ago when they struck a deal with the Icelandic government under which the commercial company would mine the health records of all 270,000 Icelanders and link genomic data with medical information (*Science*, 1 January 1999,

Since these studies took off in 2006, deCODE researchers have netted many of the more than 500 markers found for diseases such as diabetes, heart disease, and cancer. “Their research agenda has been fabulous,” says Aravinda Chakravarti of Johns Hopkins University School of Medicine in Baltimore, Maryland.

deCODE's attempts to develop drugs from these findings haven't been a moneymaker, however. The company has been in serious financial trouble since last fall. On 10 August,



Dogging
behavior

1062



LHC:
back to reality

1067

CHINA

Confronting a Toxic Blowback From the Electronics Trade

BEIJING—For Anna Leung, conducting research in Guiyu, a village in southern China where discarded computers and other electronics are stripped for their precious metals, was an assault on the senses. The acrid smell of circuit boards baking over coal fires and the stench of runoff from acid leaching were overpowering. More disturbing was the sight of children often helping their impoverished parents with the work. “We really feared for their health,” says Leung, an environmental scientist at Hong Kong Baptist University whose team found sky-high levels of toxicants in the air and soil.

Lately China has lurched from one toxic crisis to the next: Last year’s major scandal was melamine in milk, whereas the latest is the revelation that hundreds of children were sickened by lead pollution from smelters in two cities. But e-waste processing, a burgeoning cabin industry in coastal parts of China, may end up dwarfing those incidents in severity and number of victims, scientists argued at a symposium on flame-retardants here on 22 August. “The problem is just monumental,” says marine toxicologist Susan Shaw, director of the Marine Environmental Research Institute in Blue Hill, Maine. “There is extraordinary contamination of people, especially children, living in e-waste areas,” she says.

E-waste is not a new phenomenon: China has been accepting vast quantities of discarded televisions, computers, printers, and other equipment from abroad since the early 1990s. Since 2000, the central government has prohibited importation of e-waste, and a law passed last year requires e-waste processors to register with local governments and take steps to control pollution. In Guiyu, one of the biggest and most notorious processing sites in the world, banners declare that “Dealing in imported used electronics is an act of smuggling,” says Eddy Zeng, an organic geochemist at the Guangzhou Institute of Geochemistry. But because existing regulations are poorly enforced, he says, “Tremendous amounts of e-waste have been imported illegally,” such that China now processes 70% of the world’s e-waste. Much of the broken or obsolete electronics pile up in coastal villages where residents—often migrants from poorer inland provinces—use

crude methods to recover minute amounts of gold and other precious metals. One site, Longtang, is a surreal scene, says Zeng, where runoff from leaching turns streams a “very, very beautiful blue.”

The roster of substances liberated during e-waste processing is a toxicological nightmare: known carcinogens like dioxins and polycyclic aromatic hydrocarbons; neurotoxic elements like lead; and brominated fire retardants, including polybrominated diphenyl ethers (PBDEs), which have been shown to disrupt endocrine hormones in lab animals and wildlife. Zeng calculates that some 76,000 metric tons of PBDEs alone are released into the environment each year at e-waste sites in China. “This is a chemical time bomb,” he says.

The Hong Kong team, led by Ming Wong, has undertaken pioneering work to track the fate of e-waste toxicants. Along a riverbank in Guiyu, for example, they found levels of PBDE that were thousands of times higher than those found in soil from a control site in the province. “More and more of these toxic chemicals are getting into the food supply,” says Arlene Blum, a biophysical chemist at the University of California, Berkeley.

Already there is evidence that they are ending up in people. Researchers have reported that PBDE blood levels in Guiyu residents are, on average, nearly 600 parts per billion. “These are the highest PBDE levels ever reported to date in people anywhere in the world,” says Shaw. The Guiyu levels are 10 times higher than average levels in the United States and more than 100 times higher than in Europe. At Guiyu, says Leung, “villagers don’t take health precautions” such as wearing facemasks. The bottom line, says Tom Webster, an environmental scientist at the Boston University School of Public Health, is that e-waste sites “are extremely

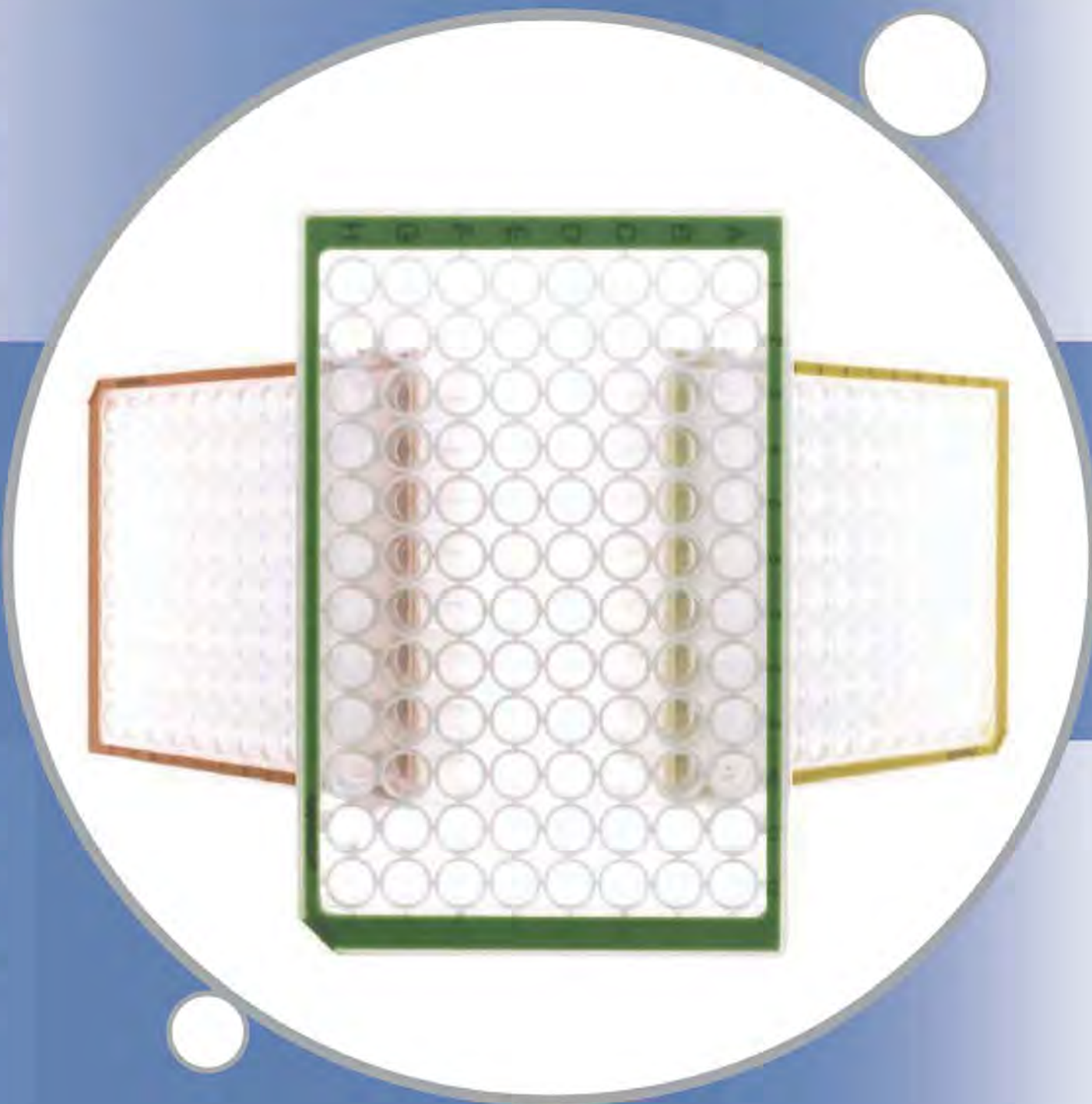


Digital detritus. Electronic waste accumulates along a riverbank in Guiyu, a world-class site of toxic residues.

good opportunities for epidemiology.”

In the meantime, scientists have proposed several broad strategies for reducing e-waste here. One approach would be to incorporate fewer toxicants into electronics. Another would be to choke off e-waste imports. China receives up to 80% of the United States’s obsolete computers, Zeng notes. “The U.S. is so generous in its contribution to China’s environmental contamination,” Linda Birnbaum, director of the U.S. National Institute of Environmental Health Sciences, says sarcastically. “This is something we should be working on.”

But China shouldn’t wait for other countries to act. One urgent priority is stricter enforcement of existing laws, Zeng and colleague Hong-Gang Ni argue in a 1 June viewpoint in *Environmental Science & Technology*. But “the problem is a lot of local agencies don’t have the resources,” Zeng says. A more promising tack, he says, might be to convince municipalities that future cleanup costs will be much greater than income from processing. And when health costs are factored in, the damage will be enormous. —RICHARD STONE



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ENGINEERING EDUCATION

Changes at Berkeley Raise Fears of Shrinking Commitment to Diversity

How can U.S. universities help more minority, women, and disadvantaged students become engineers? The University of California, Berkeley, one of the first institutions to take up the challenge, has long been a model for efforts to serve that population. But a decision by its engineering dean to overhaul programs serving underrepresented minorities has roiled the campus and triggered concern among diversity professionals throughout higher education.

"It's tough to watch because Berkeley has been a leader for so long," says Telle Whitney, CEO of the Anita Borg Institute for Women and Technology in nearby Palo Alto.

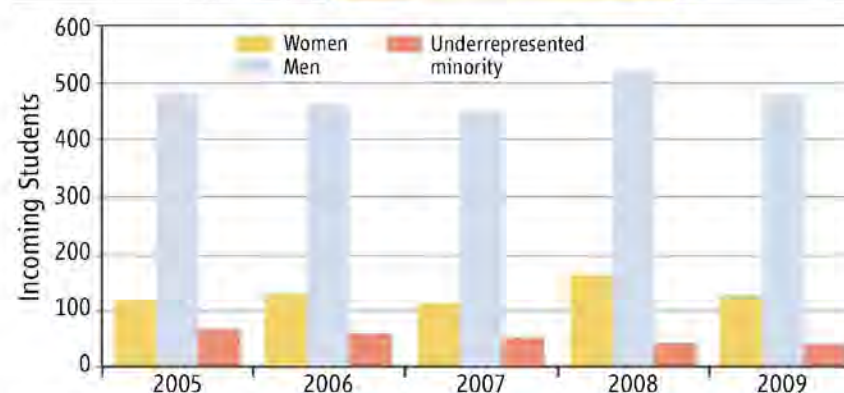
The Multicultural Engineering Program at Berkeley began in 1981 and has evolved into the present-day Center for Underrepresented Engineering Students (CUES). Its combination of early intervention, mentoring, academic and career counseling, research experiences, and other activities have helped build a sense of community among this population—and a record of achievement. "These kids come in at one standard deviation below the average but manage to graduate at the same level and go to graduate school at even higher rates," notes Stanley Prussin, a professor of nuclear engineering who oversaw the center in the late 1990s as an associate dean. "In other words, once they adjusted to Berkeley, they flew."

Many elite schools around the country have emulated that approach, which Karl Reid, an engineer who led the Office of Minority Education at the Massachusetts Institute of Technology (MIT) before becoming a senior executive last fall with the United Negro College Fund, calls a "high-touch program." But those efforts haven't improved the demographics of graduating classes in engineering over the past decade. For underrepresented minorities, it's remained flat at about 11%, whereas for women it's actually dropped from 21% to 18%.

At Berkeley, the numbers are even worse: This fall's freshman engineering class of 601 students will have 37 underrepresented minorities (defined in science as African Americans, Hispanics, American Indians, and Pacific Islanders). That 6.2% share compares with 10.4% of the 2005 class. Shankar Sastry, Berkeley's dean of engineering, is especially troubled that the yield—the percentage of students deciding to enroll in the fall after being accepted in the spring—among minorities is far lower in engineering

than in any other unit on campus.

So this summer, Sastry, who became dean in 2007, decided to take a different tack. Raise the quality of academic and career services offered to all students, he reasoned, and underrepresented groups will also benefit. Last month, he announced that CUES would merge with the college's student affairs office to form a new Engineering Student Services (ESS)



Getting a jump. Berkeley has struggled in recent years to boost the number of incoming minority and women engineering students, some of whom participate in a 2-week summer "boot camp."

office. The center's three-person staff, down from five in 2008, will lose their jobs, but ESS will be exempt from a campuswide hiring freeze and suffer no net loss of positions.

Sastry, an Indian-born electrical engineer and computer scientist who joined the Berkeley faculty in 1983, calls CUES a "fantastic program that hit a high-water mark in the late '80s and early '90s. It's done well in offering personal attention to minority students, but it's become isolated" from the rest of student services, he says. Engineering students overall are unhappy with the current level of academic and career services, he adds.

Although Sastry has vowed repeatedly to continue CUES's activities and provide more resources, many students and faculty members are not convinced. He was peppered with questions at two town hall meetings held since his 15 July announcement, and partici-

pants came away dissatisfied with his answers. Sastry agrees that the college must respond to the "special needs" of underrepresented minorities but says those students also need to be "brought into the mainstream" by experiencing all the richness of a major research university.

The center's director, Michele de Coteau, says Sastry is blaming CUES for factors beyond its control. As a public institution, Berkeley is at a disadvantage in competing against elite private schools like MIT, Carnegie Mellon University, and Stanford University that can offer targeted scholarships and generous financial aid packages. There's also California's Proposition 209, passed in 1996, which

prohibits state universities from considering race or gender in admissions. "Conflating yield and academic success just doesn't make sense," she says.

De Coteau, a 1988 graduate of the college and a Rhodes scholar who says she benefited greatly from Berkeley's programs for minorities, argues that CUES's focus on community building has paid off. "Every student needs a support group to help them believe that they belong," she says, "and to push them to do things that will foster their careers, like networking, summer internships, and scholarships for graduate school." A recent analysis shows that CUES students over the past decade have graduated at the same rate as all engineering students and that 80% of them earn degrees in science or engineering disciplines.

Ruzena Bajcsy, a professor of electrical engineering and chair of a new task force created to advise ESS, believes that better recruitment and retention of underrepresented minorities are the keys to success. CUES's supporters say the center is already working hard on those issues and that dispersing responsibility among all ESS staff will dilute the college's efforts. And whereas some people who work on diversity issues see dissolving the center as a sign that Berkeley is backing away from its historic commitment, others don't want to jump to conclusions. "The optics don't look good," says Reid, referring to the reduced visibility of what CUES had been doing. "But if the school can guarantee that it will continue these activities and provide more resources, then fine."

—JEFFREY MERVIS

With reporting by Corinna Wu in Berkeley, California.

INSECT GENETICS

Sequencing 40 Silkworm Genomes Unravels History of Cultivation

Ancient Chinese sought to protect the secrets of producing silk by executing anyone caught smuggling silkworms or their eggs out of the country. The threat worked for several millennia before the technique spread to Japan, Korea, the Middle East, and Europe. But now the Middle Kingdom is freely offering up silkworm secrets: In a paper published online this week by *Science* (www.sciencemag.org/cgi/content/abstract/1176620), a research team of primarily Chinese scientists reports on the use of modern genomic analyses to probe the domestication of the silkworm. Along the way, the group identified a host of genes likely affecting silk production, energy metabolism, and reproduction that could be exploited not only in culturing silkworms but also in raising other insects and possibly animals as well.



Size matters. Scientists are on the trail of the genes that make the cocoons of domesticated silkworms (*left*) much larger than those of their wild cousins (*right*).

Archeological evidence points to China domesticating silkworms more than 5000 years ago. But the details are elusive and still debated. Was silkworm domestication a long, slow process that gradually emerged throughout the country and its variety of ecological backgrounds? Or was it a one-time event that took place quickly and in a limited geographic area? Silkworm geneticist Zhonghuai Xiang of

Southwest University in Chongqing and genomicist Jun Wang of Beijing Genomics Institute, Shenzhen, led the group bringing modern DNA sequencing to bear on those questions. They had a reference in the complete sequence of the domesticated silkworm *Bombyx mori*, which was published in *Insect Biochemistry and Molecular Biology* last December by the International Silkworm Genome Consortium. To supplement that data, the team sequenced the genomes of representatives from 29 diverse domesticated silkworm lines from around the world, as well as 11 wild silkworms (*B. mandarina*) collected within China.

The group studied differences among the 40 genomes, identifying close to 16 million single nucleotide polymorphisms (SNPs)—locations on chromosomes where a single base varies among individuals—and other genomic variations. The data analyses and demographic modeling indicate that the domesticated and wild species are now clearly genetically separate. There is very high genetic variability in the domestic lines, though less than among the wild silkworms. From this and other genome data, the group concludes that there must have been a single domestication event that took place over a relatively short period of time during which a large number of wild worms were collected for domestication. “Whether this event was in

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CLIMATE CHANGE

Joining Forces to Pump Up a Variable Sun's Climate Effects

Plenty of past changes in Earth's climate have been pinned on an inconstant sun. Ups and downs in solar output may have triggered the Little Ice Age that gripped Europe several centuries ago, as well as droughts that brought down Chinese dynasties. But how could the slight variations scientists measure be behind such large climate events? Researchers now say two different parts of the atmosphere might be colluding to amplify the effects of even minuscule solar fluctuations.

But scientists warn against blaming every climate twitch—especially the global warming of the past half-century—on a variable sun. The evidence for a dual-pronged solar amplifier is still “on the ‘edge of significance,’” says climate modeler David Rind of NASA's Goddard Institute for Space Studies in New York City, who has modeled such sun-climate connections. And even if the amplifier exists, its

climate leverage is still relatively puny.

The latest support for a sun-climate link comes from the first study to combine two leading mechanisms for converting solar variations into climate variations in the same model. Some models operate from the top down, beginning with the few-percent changes in the sun's ultraviolet radiation that occur during the 11-year cycle of solar activity and letting them induce changes in stratospheric ozone, temperature, and circulation. Those variations, in turn, affect the climate in the lower atmosphere.

Other models work from the bottom up via a mechanism that comes into play around the equator in the Pacific Ocean. Solar energy added during the peak of a solar cycle evaporates more water vapor from the ocean. Through a long chain of changes in atmospheric and oceanic circulation, the energy-laden water vapor eventually causes fewer clouds to form in the subtropics, so even

more solar energy reaches the ocean. The result is a positive feedback loop that amplifies the climate effect of the solar variations.

Climate researchers had found that the bottom-up mechanism worked in standard climate models, which lack a realistic, ozone-laden stratosphere. The top-down mechanism produced climate effects in stratosphere models, which lack realistic ocean-atmosphere interactions. But neither mechanism alone could explain the magnitude of climate variations linked to recent solar cycles. So climate modeler Gerald Meehl of the National Center for Atmospheric Research in Boulder, Colorado, and colleagues replaced the atmosphere of NCAR's standard climate model with an atmosphere-only model that had a realistic stratosphere.

In the melded model, solar variations drove climate responses across the Pacific much like those seen during recent solar

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From *Science's*
Online Daily News Site

Why We Walk in Circles

Adventure stories and horror movies ramp up the tension when hapless characters walk in circles. *The Blair Witch Project*, for example, wouldn't have been half as scary if those students had managed to walk in a straight line out of the forest. But is this navigation glitch real or just a handy plot device? A new study finds that people really do tend to walk in circles when they lack landmarks to guide them. <http://bit.ly/3T0IHr>



Upward Lightning No Flash in the Pan

That annoying crackle on an AM radio station might not be due to a lightning strike hitting the ground. Researchers have discovered that bolts jumping from the tops of thunderclouds all the way to the ionosphere, some 90 kilometers above Earth's surface, can be just as powerful as conventional ground strikes, though they form slower. The findings suggest that thunderstorms can discharge electricity into the entire atmosphere, from Earth's surface to the edge of space. <http://bit.ly/1bfX7j>

A Pterosaur Comes In for a Landing

About 150 million years ago in what is today southwestern France, a flying reptile swooped onto a beach, landed on the damp sand, and walked off, maybe to find dinner. Now scientists have uncovered the fossilized tracks of this pterosaur, providing the first glimpse of how these flying creatures touched down. "It's a little Jurassic moment in time that got recorded," says one researcher. <http://bit.ly/VfDFv>

Where Did You Get Those Lovely Spirals?

Look at an image of the Milky Way galaxy, and you can't help but notice its exquisite spiral arms. For nearly 100 years, astronomers have tried to understand how the Milky Way and other spiral galaxies formed these dramatic patterns—and now they think they finally have the answer. <http://bit.ly/9hsiX>

Read the full postings, comments, and more on sciencenow.sciencemag.org.



Party animals. Thousands of years of cultivation has made domesticated silkworms (*left*) tolerant of crowding and human handling and unable to fly when they are moths—traits very unsuited to wild silkworms (*right*).

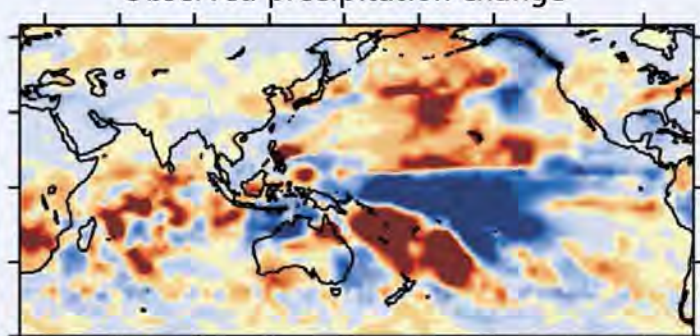
a single location or in a short period of time in several locations cannot be deciphered from the data," Wang says. They also cannot narrow down the specific region of China where domestication may have occurred.

Yutaka Banno, a geneticist at Kyushu University in Fukuoka, Japan, says the results fit in with other recent work on the relationship between the wild and the domesticated silkworms. "This paper will be accepted by almost all researchers," he says. Still, he cautions that the group has not located the origin of silk farming with any precision. He points out that only 11 wild specimens were collected and that six of those were from one province, even though the wild silkworm is found throughout China and even into far eastern Russia. "A wider survey is needed," he says.

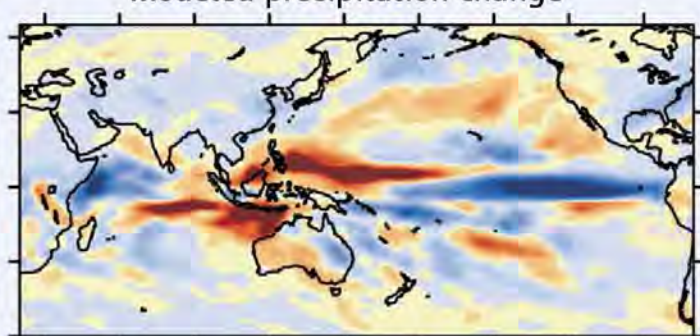
The group also compared SNP variability and other genomic features to identify DNA sequences that had been subject to selection,

ultimately identifying 354 genes possibly associated with domestication. Domestic silkworms have been selectively bred for cocoon size, growth and reproduction rates, and digestion efficiency; and Wang says some of the genes they have identified have enriched expression in the silk gland, midgut, and testis. Domesticated worms have also become tolerant of human handling and crowding while losing the ability to fly and any sense of the danger of predators, all of which renders them unable to survive in the wild. "I think it will be interesting to see whether there are any commonalities between the genes proposed to have been selected for in (silkworms) and whether any are similar to those in domesticated *higher* animals like livestock and birds, or even cats and dogs," says Marian Goldsmith, a geneticist at the University of Rhode Island in Kingston. **—DENNIS NORMILE**
With reporting by Elizabeth Pennisi.

Observed precipitation change



Modeled precipitation change



–1.6 –1.2 –0.8 –0.4 0 0.4 0.8 1.2 1.6
mm/day

maxima, Meehl and colleagues report on page 1114. The eastern equatorial Pacific cooled about as observed, and changes in precipitation also roughly resembled

Like real? Solar peak seems to increase rain (*top*, blues) on the equator and decrease rain (reds) elsewhere; hybrid model makes similar patterns (*bottom*).

observed changes (see figure). The two mechanisms reinforce each other by boosting the rising of tropical air that is driven by evaporation, Meehl explains. "That's the key commonality," he says. "That amplifies things."

Like much work in the long-controversial field of sun-climate relations, the new modeling is getting a cool reception. The study "is not nearly as conclusive as they would have it," says Joanna Haigh of Imperial College London, who developed the top-down mechanism. Among additional critiques, she and others say the researchers ran the model too few times to give reliable results. "The atmosphere and oceans are a big coupled system," she says, "but it's incredibly complicated." **—RICHARD A. KERR**

NEWSMAKER INTERVIEW

Warning: Don't Let Your Elders Brainwash You

Catherine Cesarsky has nothing against scientific road maps. The outgoing president of the International Astronomical Union (IAU) has contributed to several, including last year's report from the European astronomy consortium, Astronet, laying out a 20-year plan for European astronomy.

But Cesarsky, a former director general of the European Southern Observatory (ESO) and currently France's high-commissioner for atomic energy, believes that such documents

avoid killing each other's projects. That is particularly important in Europe, where different countries have competing interests. Astronet's road map also gives us an advantage in raising funds.

Q: What's the downside?

C.C.: You predefine what is important, what should be done, and how it should be done. I am worried that young scientists may be brainwashed as a result. It's like telling them, "Here is this primer, this cookbook—all you have to do to come up with a fundable proposal is read this well." It can do a lot of good, but it may be quenching creativity from the start.

Q: Have you seen the problem occurring?

C.C.: I do see some of it when I serve on panels for fellowships and things like that. You get proposals that are clones of each other.

Q: What's an example of a bandwagon?

C.C.: Dark energy would be one. Space missions are being planned in the U.S. and Europe to learn more about it. A lot of people want to work on the problem, and many of them are thinking along similar lines. It may be that after we have done all these experiments, we may know as little about dark energy as we know now.

Q: Hasn't it always been true that senior researchers define a set of broad research questions and that young scientists start their careers by following those lines of inquiry?

C.C.: When I got my Ph.D. [in astronomy] in 1971, things were not so well organized. All the senior people had their individual ideas. Papers had very few authors. Not everybody knew what everybody else was doing. Even Ph.D. topics were chosen a little more individually. We were, nonetheless, influenced by personalities. When I was a postdoc at Caltech [California Institute of Technology], everybody used to pay attention to what Willie Fowler thought was important.

Now we do this community work. The

documents are very well done. The Internet makes it very easy to find out what everybody is doing. Because road maps and plans are so detailed and comprehensive, it is difficult for individual scientists to come in and do better.

Q: How could the problem be solved?

C.C.: I think young scientists should guard themselves against brainwashing. They should look beyond the road maps, even if we put the best we know in them. Also, they should resist specializing too much at the cost of the big picture. The best way to escape [the] bandwagon effect is to look at things from a distance, to connect different ideas.

When I was in charge of ESO, I tried to encourage innovative projects under the director's discretionary time-allocation program. My expectation was that researchers would propose risky ideas that were completely new. Disappointingly, we got rather little of that. The program quickly became a way for scientists to add observation time to an existing project that would lead to a quick result and publication.

Q: What's your advice to reviewers and telescope time-allocation committees?

C.C.: Think again before you reject proposals that seem outlandish. When committees review ideas that go against the norm—such as pushing an instrument to its limits or trying out a new, untested method of observation—far too often they are quick to say, "Ha, that's undoable, forget it."

Q: Did you try to change that at ESO?

C.C.: I asked committees there twice a year for 8 years to be more open to unconventional projects. I don't think it has worked.

Q: Why not?

C.C.: There are often one or two individuals on each committee who are willing to take risks, but others are not. It is very difficult for a committee to not go for the sure thing.

Q: What about attaching money to scientists instead of proposals?

C.C.: I wouldn't bet entirely on the person. I like the model the European Research Council is following in awarding its new fellowships. Half the points go to the person and half to the proposal. Grantees need not do the project exactly as they have proposed, which allows room to be creative.



Pioneer spirit. Cesarsky says young astronomers should be guided as much by original thinking as by scientific road maps.

can also stifle the creativity of young scientists by forcing them down well-worn research paths. She laid out her concerns this month at the IAU meeting in Rio de Janeiro, Brazil, and elaborated on the dangers of such "bandwagon effects" in a conversation with *Science*. Her remarks have been edited for clarity.

—YUDHIJIT BHATTACHARJEE

Q: Hasn't astronomy benefited from road maps and decadal plans?

C.C.: No doubt. By having a clear set of priorities and a clear rationale for them, we

NUCLEAR TRANSPLANTATION

Researchers Prevent Inheritance of Faulty Mitochondria in Monkeys

Fusing two lines of research into egg cells and embryos, Shoukhrat Mitalipov and colleagues at the Oregon Health and Science University near Portland have achieved a technical feat that could lead to new methods for preventing the inheritance of mitochondrial diseases. Mitochondria, the source of energy in animal cells, are inherited exclusively from mothers and run on their own DNA (mtDNA). In recent years, scientists have linked numerous cancers and brain diseases to malfunctioning mtDNA, which may affect up to one in 6000 people. In a paper published online by *Nature* on 26 August, Mitalipov's team describes their success in combining nuclear transplantation with in vitro fertilization to interrupt the inheritance of mitochondrial diseases in a monkey. "I knew we could correct this, just because of the way mitochondria are passed from one generation to another, through the egg," Mitalipov says.

The goal was simple but difficult to execute.

Mitalipov sought to extract healthy nuclear DNA from an egg with mutations in its mtDNA and transfer it into an enucleated donor egg cell with healthy mitochondria. But in mature egg cells, the nuclear membrane dissolves, which makes chromosomes invisible. To locate and remove the DNA, the Oregon team developed a technique to track the spindle proteins on which chromosomes float inside cells. In addition, to transport the fragile chromosomes into the target egg, they developed a receptacle called a karyoplast, a bubble of cytoplasm barely bigger than the naked chromosomes, to which they fused the chromosomes. In theory, the karyoplast was free of mutant mtDNA, so when they transplanted it, they produced a chimeric egg with the mother's chromosomes and healthy mitochondria from the donor.

The *Nature* paper is both a technical account and a glorified birth announcement: Mitalipov and his team described the successful fertilization of the manipulated egg with sperm from a selected father, its implantation into the mother, and the delivery of twin macaques, Mito and Tracker, born in late

April. Two other macaque babies have followed. All appear healthy and hearty.

Stem cell researcher Jose Cibelli of Michigan State University in East Lansing was wowed: "I can only wish everybody could have such dexterity in the scope." Still, Cibelli's enthusiasm was dampened by the prospect of translating this bench work to the clinic. "While they make it sound easy, I am afraid it will take some time to see it implemented — if ever — in a setting other than research."

Healthy twins. Macaques born from DNA transferred into an enucleated egg.



Mitalipov says he's optimistic about adapting the technology for humans because his team consciously "mirrored the techniques used in humans for IVF treatments." He also feels the parallels with IVF and the fact that no embryos are destroyed will mitigate any ethical concerns.

Mitalipov says the work is technically germ-line gene therapy, a type of DNA manipulation that is very strictly regulated for human subjects. Questions also remain about whether the baby macaques will grow into healthy adults. The coarse sampling techniques Mitalipov used to look for mutant mtDNA could not eliminate the possibility that a few of the mother's faulty mitochondria were transplanted into the donor egg. Worse, because of the random distribution of mitochondria early in an embryo's development, this mutant mtDNA could end up concentrated in one or two organs. (Mitalipov's team did not sample tissues from all parts of the macaques, just a few.) Mitalipov says they must wait to see if the monkeys are viable adults before expanding their work to humans. —SAM KEAN

ScienceInsider

From the *Science* Policy Blog



Under intense pressure to save money, the **University of California (UC)** announced last month that all faculty and staff who receive even a fraction of their salaries from state funds will be furloughed. But researchers won't be furloughed on teaching days, according to a letter sent by UC's interim provost to faculty on 21 August.

The **novel H1N1 virus** is behaving unpredictably, U.S. health officials said at a press briefing 21 August. The virus has spread to turkeys in Chile and slowed its spread in the Southern Hemisphere. Meanwhile, drug companies are having difficulty growing the virus, which means that a vaccine will be in short supply this fall.

Research involving **human embryonic stem cells** will become easier in Japan as a result of new ethical review requirements that took effect 21 August.

The **Public Library of Science** has launched an "experimental" site for posting raw preprints of papers on hot topics. PLoS Currents (Beta) debuted last week with a set of papers on influenza.

National Institutes of Health Director **Francis Collins** has recruited a former aide to be his chief of staff. Kathy Hudson now runs the Genetics & Public Policy Center in Washington, D.C. She hopes to liaise with the FDA on overseeing genetic tests.

Top whale researchers are arguing that the U.S. National Oceanic and Atmospheric Administration should reexamine a major regulation designed to reduce collisions between ships and highly endangered **North Atlantic right whales**. NOAA says it is evaluating the regulation's efficacy.

More than two dozen pathologists have left the **Armed Forces Institute of Pathology** in Washington, D.C., to form a new company that will offer the same pathology consultation services as does the 150-year-old institute. AFIP is expected to close by 2011.

For more science policy news, visit blogs.sciencemag.org/scienceinsider.



Going to the Dogs

Cognitive scientists once spurned the dog as too domesticated to study. But now many are leaping at the chance to use man's best friend to help understand how social cognition evolved

BUDAPEST—In 1994 when Ádám Miklósi, then a young ethologist at Eötvös Loránd University in Budapest, learned that his lab's director planned to switch the team's research from fish to dogs, he couldn't believe it. "My god, are you crazy?" That's what I thought, although I didn't say it," says Miklósi, who now directs the university's highly regarded dog cognition lab. "None of us were happy about this." At the time, scientists who studied animal behavior and cognition were busy investigating a variety of species, including ants and dolphins, but they shunned dogs because they thought the animal's domestication, and the bond between human and dog (*Canis familiaris*), precluded objective study. In fact, lab director Vilmos Csányi's interest had been spurred by his admiration for Flip, a mixed-breed dog he had found in the woods and adopted. "He would tell us some crazy story about Flip and say, 'Now, devise an experiment to find out why Flip can do that,'" says Miklósi.

Fifteen years later, in the wake of dozens of provocative studies from the Hungarian lab and a few others, dogs are fast becoming the *it* animal for evolutionary cognition research. Well-known cognitive scientist Marc Hauser of Harvard University announced in February that his lab was switching from cotton-top tamarin monkeys to dogs. Around the world, from Australia to Japan, other dog ethology and cognition labs are either fully under way or in the works. Last year, Miklósi's group held the first-ever dog cognition conference; a second is planned for July 2010 at the Clever Dog Lab and Wolf Science Center (WSC) at the University of Vienna. Last month, an issue of the journal *Behavioural Processes* was devoted to the dog, with 12 papers from behaviorists and ethologists analyzing everything from canine barking to dogs' guilty faces. And this week,

Online
sciencemag.org



Podcast interview
with author
Virginia Morell.

researchers explore how dogs evolved their social smarts in a *PloS ONE* paper comparing how young and mature dogs and wolves follow human cues. Even the shape of dogs' faces is being studied: Scientists in Miklósi's lab reported 24 July in the journal *Behavioral and Brain Functions* that dogs with rounder faces, such as pugs, are better at following people's cues than breeds with longer noses, indicating that we've selected puglike breeds not for their baby faces but for their ability to look us in the eye.

Our canine pals, researchers now say, are excellent subjects for studying the building blocks underlying mental abilities, particularly those involving social cognition. The special relationship with humans that once disqualified dogs from research is now seen as worthy of study in its own right; some researchers see the dog as a case of convergent evolution with humans because we share some similar behavioral traits. And because all dogs are descended from gray wolves (*C. lupus*), they can reveal how domestication has altered a species' mental processes, enabling the dog to survive in its new habitat, the human home. "They're a natural experiment," says Josep Call, a comparative psychologist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, who studies cognition in dogs, wolves, and great apes.

CREDIT: ENIKO KUBINYI

Leading the pack. Cat lover Ádám Miklósi works with dogs in his Budapest lab.

Miklósi, Call, and others even argue that dogs may teach us more about the evolution of some aspects of our social mind than can our closest kin, the chimpanzee, because Fido is so adept at reading and responding to human communication cues. But not everyone agrees. “Completely wrong,” says Clive Wynne, a psychologist at the University of Florida, Gainesville, who argues that the skills dogs share with humans are a matter of learning rather than evolutionary change. But Wynne, who edited the special *Behavioural Processes* issue, agrees that dog ethology and cognition are hot fields. “There’s no other species on the planet that triggers so many questions and debate, and no animal that we have a more intimate relationship with, than the dog.”

Real animals?

Dogs weren’t always viewed this way. Although Charles Darwin and Ivan Pavlov considered dogs fine subjects for studying evolution and behavior, cognitive scientists and ethologists placed dogs in a kind of research purgatory for many years. In the 1970s, when cognitive ethologist Marc Bekoff of the University of Colorado, Boulder, launched a study of dog play behavior, many of his colleagues sniffed at the idea. They said “‘Why don’t you study *real* animals?’” he recalls. Domestic dogs were considered artificial and therefore “not worthy of study”—a concern that many researchers still have. Domestication makes dogs “less useful for investigations on the evolution of cognition,” says Nicola Clayton, who studies bird behavior at the University of Cambridge, U.K., “because you can’t look for the ecological effects, for what originally drove intelligence in dogs.” Also, “the investigator has no control over the nurture side: how owners feed, train, or treat their dogs,” says primatologist Frans de Waal of Emory University in Atlanta. That “introduces unknown variables we would normally not accept in animal behavior research.” Still, Clayton and de Waal support the move to dogs. When it comes to dogs, “the pros outweigh the cons,” says de Waal.

Researchers have also worried that dogs are *too* aware of human cues and so would defeat most experimenters as did Clever Hans, the horse once celebrated for tapping out answers to math problems—and later shown to be closely watching his owner for subtle clues to when he should stop tapping. To prevent this, dog ethologists carefully monitor testing methods, generally videotape experi-



Into the canine mind. Researchers are giving dogs touch-screen tests (*above*) and investigating that guilty look to understand how dogs think.

ments, and use various techniques, including blindfolding human testers, to make certain they are not inadvertently giving clues.

Dogs were also deemed to have another problem: Their brains are 25% smaller than those of their wild ancestors, wolves. “Most people thought—and some still do—that dogs were therefore not as smart as wolves,” says Call. But “brain size is not everything. ... How did domestication change wolves into dogs? Is it similar to what happened to

us? That’s the kind of thing that dogs can help us investigate.”

And despite the concerns, dogs in fact have many advantages as cognitive research subjects, say Miklósi and others. Dogs are willing and cooperative, and they enjoy being with people and following their commands. The dog’s genome has been mapped (*Science*, 21 September 2007, p. 1668), opening up the possibility of linking behavioral traits to specific genes. To cap it all off, dogs are much less expensive to study than most laboratory animals, largely because most dog labs follow the model of Miklósi’s Family Dog Project and don’t actually house dogs (which also helps keep animal-rights activists at bay). “All you need is an empty room for a dog lab,” says biological anthropologist Brian Hare, who is busy setting up such a room at Duke University in Durham, North Carolina. “Then you ask people if they’d like to have their dogs take part in a cognitive experiment. Everybody knows their dog is smart, and the next thing you know, you have 1000 dogs to test.”

“It’s like *Drosophila* genetics,” adds Hauser. “Why stop at 1000; why not have 10,000? It’s a huge change from simply studying 30 tamarins.” Thus dog research can be replicated—a tall order when working with some other animals. “Let’s face it: No one is going to be able to replicate my bonobo studies,” says Hare, “because there isn’t another population of 60 bonobos to test.”

Reading minds

With so many dog labs joining the pack, new and provocative findings are emerging. For example, ethologists have shown that dogs, even as puppies, can follow human pointing gestures to find hidden food, something difficult for chimpanzees to do (*Science*, 22 November 2002, p. 1634). The experiments suggest that dogs can read human intentions, implying that the two species share information via a complex form of communication. Even more controversially, the discovery suggests to some that dogs may understand what another being is



Raised by humans. Wolf cubs raised like dog puppies (top) play with dogs and grow up to be as good as adult dogs at following human pointing cues in experiments.

thinking, a sophisticated talent called theory of mind that many researchers think even chimpanzees and preverbal human infants lack.

Dogs can also imitate a human's actions on command ("Do as I do!"), much like children playing "Simon Says," according to studies done by Miklósi's colleague József Topál and his team at the Institute for Psychology at the Hungarian Academy of Sciences in Budapest. That's an indication that dogs are strong social learners, something still debated for chimpanzees. "They've been selected for this sensitivity and to cooperate," says Miklósi, who confesses to being a cat person and has never owned a dog. Dogs can also use computer touch-screens to demonstrate some glimmerings of abstract thought, such as the ability to form a concept. For example, dogs were able to select color photographs of other dogs instead of landscapes, choosing with a quick nose-touch to the screen. Pooches can follow human rules, a social skill that helps strengthen group bonds; they have a sense of fairness; and some canine whizzes—all Border collies so far—have vocabularies of several hundred names, suggesting an ability to learn words that some say resembles that of infant children. Also like human toddlers, dogs willingly imitate another's actions, even if these don't always

make sense, an ability that makes it easy to learn from others. In short, dogs are skilled at cognitive tasks, especially social tasks requiring cooperation and sharing information to achieve a goal.

Sometimes these advanced social skills lead dogs to behaviors and emotions that seem very humanlike. But are they? Take the "guilty look" every dog owner knows. While cameras rolled, Alexandra Horowitz, an animal cognition researcher at Barnard College in New York City, had 14 owners show their dogs a tasty treat and tell them not to eat it. But when the owner left the room, Horowitz either gave the dog the forbidden food or removed it, making it impossible for the pooch to misbehave. Later, some owners were told that their dogs ate the treat, even when they had not. "It didn't matter if the dogs had done the right thing. If they were scolded, they showed that guilty look," slinking away with their heads drooping and ears flattened, says Horowitz, whose work is published in the July issue of *Behavioural Processes*. "And the dogs who showed the most 'guilt' were the ones that hadn't disobeyed. We've trained them to give us that look" in response to our anger. "The guilty look is not necessarily a reflection of anything they have done," she says. Adds Michael Tomasello, a comparative cognition researcher also at the Max Planck Institute in Leipzig: "Guilt involves a moral dimension, which dogs very likely don't have. But by showing guilt, we may lessen our punish-

ment. What people see in dogs is the anticipation of punishment." In humans, says Tomasello, that anticipation is regarded as a "precursor to guilt."

Indeed, some researchers think that the evolutionary steps gray wolves took to live in human society may in some ways mirror the transition from ape to human. The loss of fear and aggression toward others and the emphasis on cooperation rather than competition are steps that early hominids must also have taken as they organized into groups, although for dogs the change also required evolving a deep attachment to an entirely different species. "Dogs have lived with us for at least 10,000 years, and they've evolved to fit into our social world, so they've developed some social and behavioral skills strikingly like ours. It's a case of convergent coevolution," says Call.

Wynne's having none of the coevolution argument, however. "Dogs and wolves are still the same species," he says. "They still interbreed, and so any changes in the dog's brain are a matter of degree rather than kind." And he is skeptical of some of the claims regarding dogs' abilities, particularly those that suggest superior mind-reading skills as compared with wolves. "Sometimes I feel that I'm always running after other people, stomping out the fires they've started" with claims of dogs' advanced abilities, he says. He argues that the Border collies with fat vocabularies are merely "conditioned" and that their talent for picking up human sounds is not at all comparable to

CREDITS (TOP TO BOTTOM): COURTESY OF FRIEDRIKE RANGE, WOLF SCIENCE CENTER; ENIKO KUBINYI

how children learn words. “If they were really like children, they’d be learning hundreds of words a day,” he says.

From wolf to dog

Despite Wynne’s concerns, for many the evolutionary process that produced the dog is fertile ground for research. Most agree that domestication changed the dog dramatically, turning it into a creature who yearns to be with a species other than its own. Researchers hypothesize that this happened because humans selected dogs for their ability to socialize and to form strong attachments with people. Indeed, experiments in Miklósi’s lab have shown that 4-month-old puppies in a choice test always preferred a human companion to a dog. Young wolves showed no preference. “Domestication changed” the dog brain, “making it more attuned to human social signals,” says Miklósi.

To test that idea, scientists have examined how well dogs and wolves can pay attention to human pointing cues and hold eye contact with people. (Wild wolves, like many wild mammals, are reluctant to make eye contact, perhaps because in many species staring is a threat.) Previous pointing studies had mixed results: In the original tests, dogs proved to be superior to wolves (and chimpanzees) in following a human’s finger to a bucket that held a hidden food treat. Many researchers interpreted this as an indication of a genetic component to this skill, one favored by domestication. But later tests complicated the issue: In another study using a different method, wolves actually topped the hounds.

To clarify the question of just what wolves can do, Márta Gácsi of Miklósi’s lab and her co-authors hand-raised gray wolves much as pet dogs are raised, as they report this week in *PLoS ONE*. Then they gave carefully designed pointing tests to wolves and dogs of different ages. Intriguingly, 8-week-old and 4.5-year-old wolves were as good as the dogs at following the human pointing cues to hidden food. But the 4-month-old wolves failed. “They were struggling and biting; they were just too busy doing other things,” says co-author Friederike Range of WSC. In addition, in contrast to the young dogs, the young wolves had trouble making eye contact with their humans. “They are on a different developmental path from dogs,” perhaps because ultimately dogs “must live in our world and obey our rules. They have to learn many of the same things that children learn,” says Range. And so it behooves dog puppies to look into their owners’ eyes and pay attention.

“The study is a slam dunk,” says Hare, who says it shows “once again that dogs and wolves are different.” He and others say that the study reveals that dogs are born ready and willing to work with people, whereas wolves face a long learning curve before they can accept two-legged creatures as social partners. But because the wolves *can* learn over their lifetime to follow our cues, the study also points out how difficult it is to “unravel genetic factors” from socialization and training, notes de Waal.

“We have so many questions to ask,” says Miklósi. “What do dogs understand about verbal commands? How do they recognize their owners? What do they think of as sig-

warnings or to protest. In contrast, dogs bark for many reasons, says Miklósi. “They ‘invented’ barking,” as a means of communicating to us as much as to other dogs, and they “can modulate the frequency and pulse” to signal fear or that they are feeling lonely or playful. In a previous study, Peter Pongrácz in Miklósi’s lab showed that humans can readily identify the differing barks of a lonely, fighting, or playing dog. “That means that a dog’s bark is often directed at us to convey the dog’s inner state,” Pongrácz says, an ability that has likely come about because dogs live with a talkative species.

As part of his ongoing, as-yet-unpublished

study of dog-human communication, Tamás Faragó, one of Pongrácz’s graduate students, covered a small cage with a cloth and placed a large, tempting bone close by. Kope, a bright-eyed Cairn terrier, trotted in off-leash with his owner, spied the bone, and made a beeline for it. But just as Kope reached the bone, Faragó played a recorded growl, and the dog froze in place. “That’s a food-guarding growl,” Faragó whispered. “As soon as a dog hears that, he knows he better leave that bone alone.” When the unseen dog growled a second time, Kope, looking unnerved, ran halfway back to his owner, wagging his tail. He peered up at her face, then looked back over his shoulder at the bone. “He’s asking for help,” Faragó explained. “‘Come on, Mommy, help me get that bone. Let’s do it together.’”

That, say Miklósi and others, is what lies at the heart of dog cognition: their strong desire to work with and for us, and their ability to communicate with us without language. “That’s the one thing all of us doing dog research must never forget,” says Tomasello. “Dogs are collaborating with us; they aren’t doing this with other dogs.” Unraveling how that collaboration came about—how it turned wolves into dogs, and humans into dog lovers—is like a good bone: one well worth digging for.

—VIRGINIA MORELL



The changeling. Domestication turned gray wolves into cooperative dogs attuned to human social cues.

nificant in their environment? What does a dog understand about human relationships; what does he know about your state of mind? And then we should ask the wolves the same questions.”

On one recent day, dogs and their owners stopped by to join in an experiment at the Hungarian lab, investigating how dogs respond to certain growls, part of a larger study aimed at understanding how dogs’ barks and growls have changed from those of wolves. Wolves bark, too, but only as

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Steady ... Technicians repairing the LHC ease a massive superconducting magnet into place.

PARTICLE PHYSICS

The Large Hadron Collider Redux: Hoping for a Long, Hard Slog

After spending a year on repairs, accelerator physicists hope the revamped LHC will finally come to life more as its predecessors did—in a steady stream of smaller problems and headaches

In 1991, as accelerator physicists at the Deutsche Electron Synchrotron (DESY) laboratory in Hamburg, Germany, turned on their new \$650 million particle smasher, they ran into some technical glitches. Researchers found one problem in the circuits meant to protect the superconducting magnets at the heart of the 6.3-kilometer-long Hadron Electron Ring Accelerator (HERA) from destroying themselves in an emergency. But in trying to fix the problem, they made it worse, recalls Karl Hubert Mess, then a DESY staff member.

To diagnose the problem, Mess and colleagues disconnected part of the circuit. But that overloaded a key element called a cold diode. “We were stupid. We disconnected the protection” for the diode itself, Mess says with a sheepish smile. Researchers burned out two or three diodes before realizing their mistake, says Mess, now a staff member at the European particle physics laboratory, CERN, near Geneva, Switzerland. Replacing each one took a week, as it required warming part of the massive machine from near absolute zero to room temperature.

It’s a cautionary tale for physicists awaiting the restart of CERN’s Large Hadron Collider (LHC), the world’s highest-energy atom

smasher, which wrecked itself last fall just 9 days after it started up. Accelerator physicists at CERN and elsewhere doubt the LHC will suffer another catastrophic failure. But they expect researchers working on the 27-kilometer-long, \$5.5 billion machine to run into smaller problems, like those that dogged DESY. And given the immense size of the LHC, fixing them may take much longer.

CERN researchers have modified the LHC to prevent a repeat of last year’s fiasco, in which a splice in a superconducting electrical line between magnets melted, setting off a chain of mechanical failures (*Science*, 26 September 2008, p. 1753). Researchers have also found additional faults in the copper cladding around those splices (*Science*, 31 July, p. 522), so to ensure the junctions’ safety, they will initially blast protons together at half the energy the machine was designed to achieve (*Science*, 14 August, p. 799). Challenges remain, but “CERN is incredibly powerful intellectually,” says Stephen Peggs of Brookhaven National Laboratory in Upton, New York. “Nobody in their right mind would bet against CERN.”

In fact, given the way it purred before breaking down, accelerator physicists generally expect the LHC to start smashing parti-

cles without much ado. However, they say it will be much harder to reach the machine’s design goals—collisions at an energy seven times higher than has been achieved before and a 30-fold increase in the record for the rate of collisions, or “luminosity.” The three accelerators most like the LHC—HERA, the Relativistic Heavy Ion Collider (RHIC) at Brookhaven, and the Tevatron Collider at Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois—took years to reach their design goals. And some accelerator jocks wonder whether the LHC will ever reach its outsized specs.

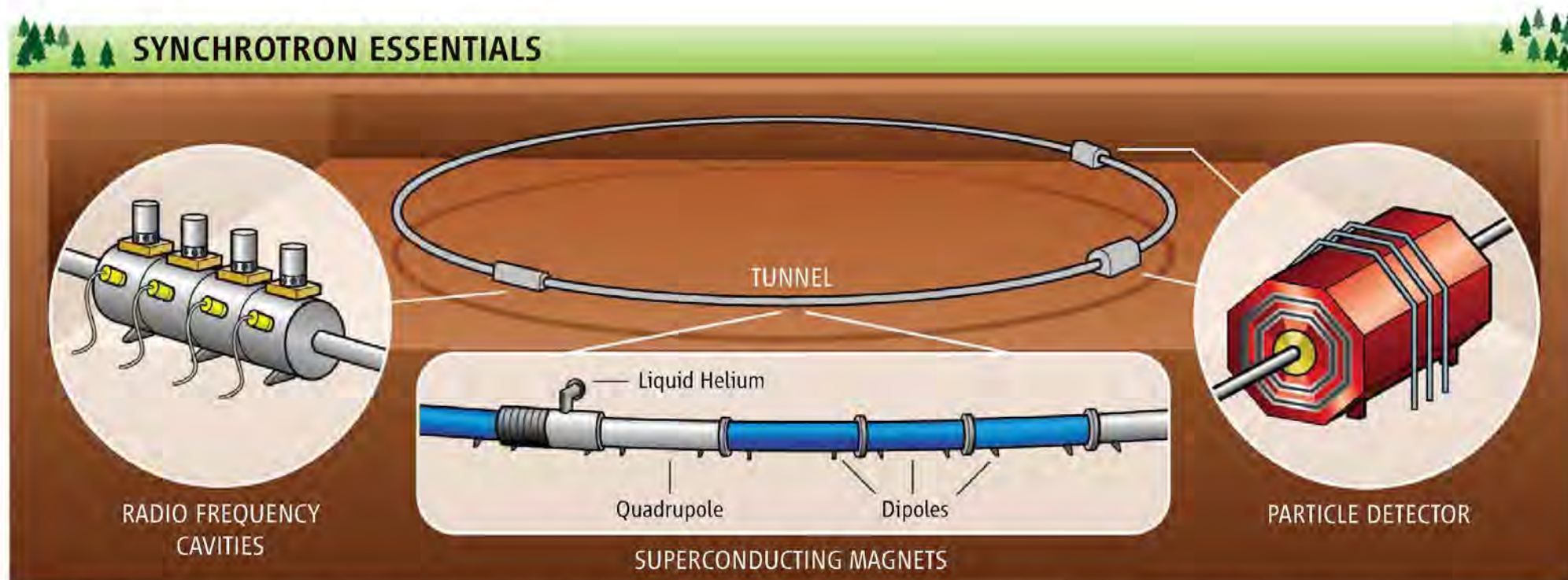
“I’m confident that if they don’t have any more of these electromechanical problems, then they’ll get some usable luminosity and get up to a fairly high energy fairly rapidly,” says Fermilab’s Peter Limon. “And then it’s going to be a struggle to get to design luminosity and design energy.” Brookhaven’s Michael Harrison, who directed the construction of RHIC, says “there will be a problem, and you’ll solve it. And then there will be another problem. You fight your way up.”

An explosive mix of technologies

The LHC and its kin are circular raceways for subatomic particles known as superconducting synchrotrons. Accelerating particles to near light speed, such machines are ideal for smashing them to make exotic states of matter, blast out new particles, or even search for new dimensions of space. In a synchrotron, electrically charged particles circulate through an evacuated beam pipe. Each time they pass through powerhouses called RF cavities, the particles receive a boost from oscillating electric fields within them (see diagram, p. 1068). As the particles gain energy, electromagnets called dipoles bend their trajectory to keep them from flying out of the machine. Meanwhile, magnets called quadrupoles focus the beam.

In a superconducting synchrotron, superconducting magnets replace conventional ones, which consist of copper wire wound around iron “pole pieces.” Conventional magnets suck up lots of power, and the magnetic properties of iron keep them from producing fields above 2 tesla—40,000 times Earth’s field. Because current flows through superconducting magnets without resistance, they consume much less power. And they have no iron poles, so they can reach much higher fields—8.3 tesla in the case of the LHC magnets. “This makes a collider much smaller and saves a lot of moola,”

SYNCHROTRON ESSENTIALS



Floor it. RF cavities accelerate particles, and magnets guide them around the ring. The beam can be used to generate collisions within particle detectors.

Limon says. Compactness was key for the LHC because it had to fit into a tunnel dug for an earlier collider.

But the advantages come at a price. The superconducting alloy used in the magnets, niobium-titanium, carries current without resistance only at temperatures within 9 kelvin of absolute zero. So the hearts of the magnets must be bathed in frigid liquid helium, and an accelerator must have huge cryogenic plants to crank out the stuff. Moreover, if the magnets overheat—perhaps because stray particles hit them—they can suddenly lose their superconductivity, or “quench.” If they do, automated quench-protection systems must quickly shunt thousands of amps of current out of the magnets before they fry themselves.

The combination of cryogenics and accelerator technology paves the way for new modes of failure, as last fall’s accident at the LHC demonstrated. Researchers were ramping up the current in a string of 154 of the machine’s 1232 dipoles—each 15 meters long and weighing 35 metric tons—when a faulty electrical splice between two magnets melted, sending 9000 amps of current arcing through the machine.

Like a welder’s torch, the current cut the tube that kept the line bathed in helium. The breach sent boiling liquid pouring into the magnets’ evacuated casings at a rate of 20 kilograms per second, says Francesco Bertinelli, a mechanical engineer at CERN. Because researchers had not foreseen such a deluge, he says, the magnet casings had emergency relief valves designed to cope with only 2 kilograms per second. So a pressure wave blasted through the magnets, damaging 53 of them and ripping some from their moorings.

Some accelerator physicists say CERN researchers dropped the ball by not designing

the LHC’s quench-protection system to adequately monitor the tiny voltage across each splice, which spikes as a splice heats up. “It was an honest-to-God screw-up,” says Brookhaven’s Harrison. CERN researchers have now installed a much better system. “You can ask why didn’t we do it earlier,” Bertinelli says. “Well, ... we should have.”

Troublesome loose ends

To some physicists, the LHC mishap is vaguely reminiscent of the travails of the first superconducting synchrotron, Fermilab’s Tevatron. Conceived in the 1970s, the Tevatron was still being designed even as it was being built in the early 1980s, recalls Fermilab’s Helen Edwards, who oversaw the machine’s commissioning. “The Tevatron was peculiar in that the first sector of the machine was rebuilt a number of times before we got to a point where we could consider the design set,” she says. Nevertheless, the Tevatron came to life in 1983 fairly smoothly, providing a beam of protons to be fired into “fixed target” experiments.

After about a year, however, the connections between the Tevatron’s 774 dipole magnets started exploding, recalls Fermilab’s John Peoples Jr., who led the effort to turn the Tevatron into a collider. The explosions happened because the superconducting leads connecting one magnet to the next were supposed to be tied down, but many were not. When the current in the leads ramped to 4000 amps, magnetic forces made the stiff leads flex and eventually break. “By the time the quench-protection system figured out what was going on, the leads had already evaporated,” Peoples says.

After losing five magnets in 4 months, physicists shut down the Tevatron in July 1984 to secure the leads. The problems eventually

reemerged, and workers repeated the repairs in 1988 and 1989. Still, the machine continued to progress. In September 1985, researchers circulated protons and antiprotons in opposite directions in the machine’s single-beam pipe to make it a collider. After a yearlong shutdown, the Tevatron started providing collisions for experiments in February 1987, reaching its design luminosity a year later. Fermilab researchers benefited from their ability to warm up a part of the machine, replace a magnet, and cool it down in several days. That process takes far longer for the massive LHC. “At CERN, it’s at least 2 months. That’s a big deal,” Peoples says.

In contrast to the Tevatron, HERA never lost a magnet, and Brookhaven’s RHIC hasn’t so far. But both went through a lengthy start-up phase. In its first data run in 1992, HERA cranked out collisions between protons and electrons at only 0.14% of its design luminosity. That figure climbed to 10% a year later. “It took us until 1995 to get up to something close to design specs,” says Ferdinand Willeke, who oversaw the commissioning of HERA’s superconducting proton ring and now works at Brookhaven.

RHIC came up more quickly. After a short trial run in 1999, it began smashing gold nuclei together for nuclear physics experiments in early 2000 and reached its design luminosity for brief periods by the end of 2001. Even so, of the 167 days RHIC ran in its first full year, only 38 were spent providing collisions for the four experiments the accelerator fed. The rest were given over to working on the accelerator itself.

The LHC’s predecessors took different amounts of time to reach their energy goals. HERA, which shut down in 2007, came on at design energy; RHIC reached it in 2 years. Because of design changes, the Tevatron took

data for 9 years at 80% of its design standard of 1 tera-electron volt per beam for a total collision energy of 2 TeV. A major upgrade begun in 1996 and finished in 2001 pushed that up to 98% of design energy, or 1.96 TeV.

Getting (re)started

The LHC won't hit its design goals right away, CERN researchers acknowledge. It's built to blast protons together at 30 times the rate currently achieved by the Tevatron, and learning how to cram enough particles into the accelerator to do that will take time. "We're working on the assumption that we will get there in 5 years," says CERN's Lyndon Evans, who directed the LHC's construction. The LHC will need 2 or 3 years to reach design energy, Evans says. If it does, it will accelerate each beam to 7 TeV for a total collision energy of 14 TeV—more than seven times the Tevatron's collision energy.

To start that long climb, physicists first have to get the LHC up and colliding particles. CERN officials plan to start that process in mid-November. First, they must inject protons from a lower-energy accelerator and coax them all the way around the LHC's two beam pipes, one for protons circulating in each direction, which run in parallel through the same magnets.

Researchers must gain control of the beam and increase the number of laps it will make until they can hold on to it indefinitely. Next, they must "capture" the beam with the oscillating electric fields produced in the RF cavities. Only then can they begin to accelerate the particles and increase their energy. Once they have two beams circulating stably at the desired energy—at 3.5 TeV to start with—physicists can steer them to collide in the centers of the four particle detectors spaced around the subterranean ring.

In fact, CERN researchers accomplished much of this work last fall. Between sending beams around both rings on the first try on 10 September and the accident 9 days later, they circulated beams for a total of just 60 hours. But in that short time, physicists managed to control one beam and catch it with the RF cavities. "The kind of things that took them a few days took us a lot longer, on the order of weeks," says Brookhaven's Harrison. Given that virtuoso performance, he says, this year "it's conceivable that they could have collisions within a week."

But others say that increasing the energy of the beams may be tricky. That's because the current in the dipole magnets has to ramp up at the same time, and as the magnets' power supplies pour on the juice, that very act induces small, hard-to-predict eddy currents. Those

How to Make a Collider

A synchrotron circulates particles in one direction, so it takes some extra work to make one into a collider. One way is to set particles and antiparticles circulating in opposite directions within a single synchrotron, as is done with protons and antiprotons at the Tevatron at Fermi National Accelerator Laboratory in Batavia, Illinois. Another scheme that requires no antiparticles is to build in one tunnel two synchrotrons that send the same particles in opposite directions, as is done in the Relativistic Heavy Ion Collider at Brookhaven National Laboratory in Upton, New York. The Large Hadron Collider at the European laboratory CERN near Geneva, Switzerland, is really two synchrotrons in one, with two beam pipes running through a single set of special "2-in-1" magnets. It will smash protons into protons.

—A.C.

fleeting currents create additional fields that may disrupt the beams. To compensate for such "snapback," the accelerator's control system must adjust special sextupole magnets at the same time. Evans says CERN's system is up to the task, but other researchers say the energy ramp is the thing to watch this winter.

Once CERN researchers achieve collisions, reaching design luminosity may be difficult. In part, that's because LHC's full beam will contain so much energy—350 megajoules compared with the Tevatron's 1 megajoule—that if a tiny fraction hits a magnet, it will quench it. "The LHC lumi-

dipoles seem to have forgotten theirs. Retraining them in place is time-consuming, so the problem could limit the LHC's energy to about 6 TeV per beam, says Fermilab's Limon. "Personally, I wouldn't worry about it," he says. "Six TeV is pretty good, and it's not like anybody is competing with them."

Broken fingers and ice balls

As they work their way up, CERN researchers are bound to run into unpleasant surprises. For example, when physicists at Brookhaven turned on RHIC in 1999, they found that a piece of metal in an expansion joint in the beam pipe had bent into the path of the beam. Like commuters easing around a stalled car, researchers simply steered the beam around the obstructing "RF finger" until it could be removed later.

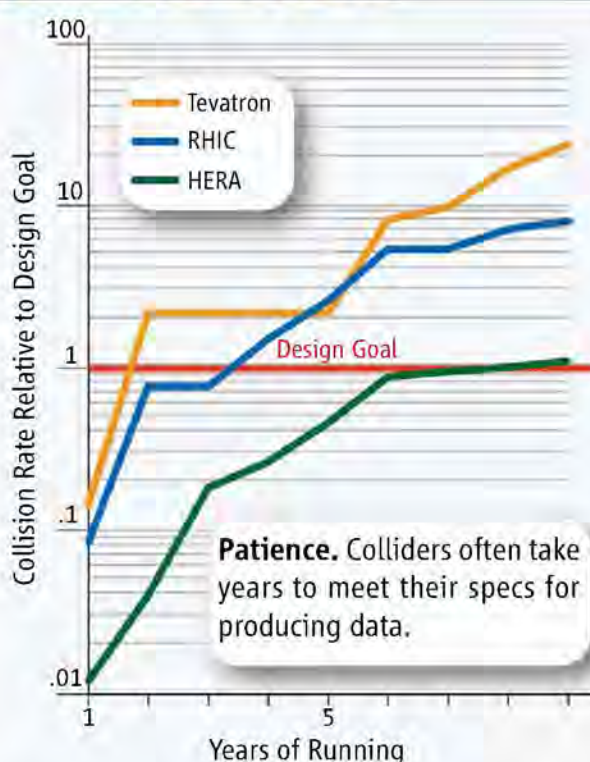
Researchers must constantly balance the need to get subsystems up and running against the risk of pushing too fast and creating more problems, says Fermilab's Edwards. "It's all judgment," she says. "You're going to make mistakes for sure."

At least early on, most of those judgments will be made in a blizzard of minor crises. Brookhaven's RHIC has never suffered a catastrophic failure; nevertheless, during its first year of running, scientists and technicians had to idle the machine and enter its tunnel to fix something more than once a day, Fischer says. Often, they had to restart a power supply. Sometimes Fischer and others had to melt ice balls that would form on poorly insulated parts of the machine.

The repairs made for hectic days and long night shifts in the accelerator's control room. "I didn't do much else," Fischer says. "It was pretty much working, eating, sleeping." Yet, he adds without hesitation, "It was some of the best time of my professional life." Bringing the LHC to life will likely entail years of battling one little technical problem after another. Instead of blanching at the prospect, accelerator physicists relish the challenge.

—ADRIAN CHO

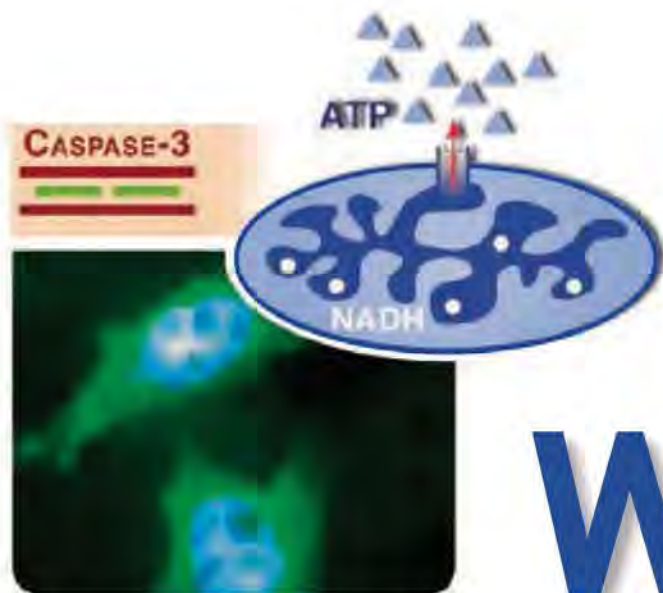
COMING UP TO SPEED



luminosity is very challenging," says Brookhaven's Wolfram Fischer. "They have really tried to push every beam parameter to the limit."

Others question whether the LHC can reach its design energy. The stumbling block they foresee isn't the junctions between magnets—those will be fixed, probably in winter 2010—but the magnets themselves.

To go to high fields, a magnet must be trained—allowed to quench at lower fields. Magnets are supposed to remember their test-stand training, but dozens of LHC



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Apoptosis has been implicated in a broad spectrum of biological processes. As our understanding of the apoptotic process has expanded, it has become clear that it is a fundamental means by which organisms shape an appropriate multicellular architecture and maintain homeostasis and normal function. Researchers have shown that proper regu-

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LETTERS

edited by Jennifer Sills

Influenza: Accounting for Prior Immunity

IN THEIR REPORT "PANDEMIC POTENTIAL OF A STRAIN OF INFLUENZA A (H1N1): EARLY FINDINGS" (19 June, p. 1557), C. Fraser *et al.* analyzed the spread of novel A/H1N1 influenza in Mexico. They reported values for the basic reproduction number in a fully susceptible population (R_0) in the range 1.2 to 1.6. It would have been preferable to instead report these as values for the effective reproduction number given prior immunity (R). The authors acknowledge that cross-immunity may lead to increased R_0 estimates. However, by conflating R_0 and R , they effectively assume that with a new virus, the entire population is susceptible. We do not believe that this assumption is necessarily justified.



Influenza A H1N1 (2009) virus

We do not believe that this assumption is necessarily justified.

In the 1918–1919 pandemic, sequestered populations in boarding schools (1) and isolated communities in Alaska (2) had much higher attack rates than air force bases in the United Kingdom (1) or urban populations (3), arguably because isolated populations had little prior immunity from regular exposure to seasonal influenza. Likewise, attack rates were high in the isolated island of Tristan da Cunha when H3N2 influenza first

arrived in 1971 (1). Detailed analyses strongly suggest that the differences between isolated and non-isolated populations can be explained by differences in prior immunity and hence effective R (1, 4).

Likewise, the higher attack rate found by Fraser *et al.* for children (61%) compared with adults (29%) in La Gloria village in Mexico could be due to lesser prior immunity because of fewer exposures to seasonal influenza.

In the longer term, the degree of difference between R_0 and R could be of considerable practical importance for non-isolated populations facing a new pandemic, given that our new analyses of 1918–1919 data suggest that prior immunity waned over a period of several months (4). If the same thing happens in 2009, this could leave populations exposed to an effective R that approaches R_0 .

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4. J. D. Mathews *et al.*, paper presented at the International Workshop on Modelling and Data Analysis for Infectious Disease Control, South Durban, Australia, 9 to 12 March 2009.

Influenza:
H1N1 Goes to School

A KEY DETERMINANT OF THE SUCCESS OF influenza containment is the transmission rate of the novel strain. C. Fraser *et al.* ["Pandemic potential of a strain of influenza A (H1N1): Early findings," Reports, 19 June, p. 1557] estimated the basic reproduction number (R_0) of the Mexican outbreak of influenza A (H1N1) to be in the range of 1.2 to 1.6. The value of R_0 is a key measure of transmissibility and estimates the number of secondary cases in a completely susceptible population. Their findings were comparable to lower estimates for the 1918 pandemic, where R_0 ranged from 2 to 3 (1).

To further investigate the transmissibility of this novel virus, we conducted a secondary analysis of the largest reported cluster of influenza A (H1N1) (2). We used survey data from students of the St. Francis Preparatory School outbreak in the United States to calculate the effective reproduction number (R) in a school-based setting. R is the average number of secondary cases generated by an infectious case during an epidemic and is usually comparable to R_0 . This survey collected data on self-reported fever and either cough or sore throat between 8 and 28 April 2009.

We used the method proposed by Vynnycky *et al.* (3) to calculate R from the growth rate of the epidemic. We based our parameter assumptions on estimates for seasonal influenza commonly reported in the literature, because such values are not yet available for H1N1. The parameters were as follows: incubation period of 2 days; infectious period of 3 days; and a calculated serial interval of 5 days. The serial interval is the time between the onset of symptoms for first and second generation cases. Using daily data from the outbreak growth phase, we calculated R to be 2.69 [95% confidence interval: 2.20 to 3.22; degrees of freedom (df) = 13]. Increasing the estimated infectious period to 5 days results in an R of 3.45 (95% confidence interval: 2.74 to 4.28; df = 13). The confidence interval

for R was derived from a Monte Carlo simulation based on the uncertainty of the slope estimate. Estimates of R were relatively insensitive to the use of data from the growth phase or entire outbreak.

Our calculated R is specific to this school setting and, with the increased transmission potential of a close-contact setting such as a school, is likely to be higher than transmission rates in the community-wide Mexican outbreak. The use of parameters estimated from seasonal influenza will need confirmation for the 2009 influenza A H1N1 virus. Our analysis supports the findings from Fraser *et al.* that this H1N1 virus has a transmission rate comparable to the lower R_0 estimates of 2 for the 1918 pandemic (1).

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Influenza: Making Privileged Data Public

IN THE REPORT BY C. FRASER *ET AL.* ["Pandemic potential of a strain of influenza A (H1N1): Early findings," 19 June, p. 1557], the authors used data, collected by their own agency, that had not been made public. It is not uncommon for researchers to have access to privileged data from government agencies; agencies have the responsibility to handle sensitive information that might be misused or misrepresented, and they collaborate with trusted scientists, often staff researchers. However, this raises a potential conflict of interest, given that staff researchers or officials might use their public authority to withhold information for the sole purpose of publishing a paper. I believe that the following criteria should serve as guidelines to make public and scientific use of data more transparent.

There should be a distinction between privileged data obtained by publicly funded scientific research and data obtained by acts of government. The latter raises ethical questions as to if and how the agency's personnel should be able to author scientific papers

using the data. If they do want to author a scientific paper, therefore acting as researchers and not officials, they should do so only with information that has already been released to the public. If the information cannot be released to the public, they should abstain from authoring or sign as the whole agency, not individual coauthors.

The case of the Fraser *et al.* paper made this dual role evident; the coauthoring Mexican Ministry of Health officials released results of their analysis in their capacity as researchers but withheld the data that the paper analyzed in their capacity of public officials. It is easy to interpret these actions as an honest mistake, but can also raise questions of conflict of interest. Had the authors followed the criteria mentioned above, the controversy would not exist.

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Response

WE THANK MCCAW *ET AL.* FOR THEIR COMMENTS. Cross-immunity between new pandemic strains and preexisting influenza

viruses is an intriguing possibility that has been explored in the context of explaining inter-subtype competition in pandemics (1). Indeed, preliminary published data suggest limited cross-reactive immunity to the novel H1N1 virus in the elderly (2). In terms of the relative merits of referring to the basic versus the effective reproduction number (R_0 versus R), we used R_0 because we were describing the spread of a novel influenza virus in a large population that had not been previously exposed to this virus strain. R_0 is a population-specific composite variable (not a fundamental biological parameter) and is only well-defined in a large population; there are examples of higher attack rates in past pandemics in close-contact closed communities, but these were not representative of spread in large populations. Variation in attack rates by age may indeed suggest age-dependent susceptibility, but this was not caused by previous exposure to this novel virus and could equally be due to innate immunity or other biological mechanisms. We therefore felt that it was appropriate to describe spread using the term R_0 . However, we agree that the possibility of exposure to previous subtypes conferring partial immunity to the new H1N1 virus may—together with antigenic evolution—have implications for transmission of the pandemic virus over the coming months.

CORRECTIONS AND CLARIFICATIONS

Letters: "How to improve U.S. education" by F. J. Rutherford (7 August, p. 674). The author's address is 1809 8th Street, Berkeley, CA 94710, USA. Also, reference 8 was incorrect. The author is a former Chief Education Officer of AAAS.

Reports: "Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects" by R. Rupprecht *et al.* (24 July, p. 490). In the fourth line of the caption for Fig. 2, the dosage of XBD173 should be: 0.1, 1, or 10 mg/kg orally.

Reports: "Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins" by Y. Tada *et al.* (15 August 2008, p. 952). In the title, the word "Changes" was incorrectly printed as "Charges."

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Floral Iridescence, Produced by Diffractive Optics, Acts As a Cue for Animal Pollinators"

Nathan I. Morehouse and Ronald L. Rutowski

Whitney *et al.* (Reports, 2 January 2009, p. 130) investigated the mechanism of iridescence in hibiscus and tulip flowers and suggested that bumblebees are able to use this iridescence as a pollination cue. However, their study failed to isolate iridescence from other coincident visual cues, leaving open questions regarding the importance of iridescent stimuli in foraging-based associative learning in bumblebees.

Full text at www.sciencemag.org/cgi/content/full/325/5944/1072-d

RESPONSE TO COMMENT ON "Floral Iridescence, Produced by Diffractive Optics, Acts As a Cue for Animal Pollinators"

Heather M. Whitney, Mathias Kolle, Piers Andrew, Lars Chittka, Ullrich Steiner, Beverley J. Glover

Morehouse and Rutowski make interesting comments on the difficulties of untangling complex optical phenomena. However, our use of a four-colored transfer test in our original study, along with spectrophotometric analysis of the nonoverlapping colors produced by our target disks, allows us to conclude that bees can learn to use iridescence as a foraging cue.

Full text at www.sciencemag.org/cgi/content/full/325/5944/1072-e

Paterson *et al.* contribute interesting additional data to inform our understanding of this virus. As noted above, reproduction numbers calculated from close-contact institutional settings (such as schools) are known to be substantially higher than community average values. In addition, there may be a selection bias for larger initial growth rates given that schools with imported cases but little secondary spread may have been overlooked. Estimates of R_0 derived from epidemic curves are highly dependent on assumptions made about the generation time distribution (determined by the incubation period and duration of infectiousness) (3). Our current analyses indicate that a mean generation time of about 2.8 days is appropriate for this virus. This is roughly consistent with estimates for seasonal influenza derived from transmission studies (3–5) or viral shedding data (5, 6). Use of this value would suggest a somewhat lower R_0 value of about 2 for the St. Francis school outbreak.

Ormsby and Reyes-Terán raise a set of interesting issues. Before the publication of our Report, the Mexican data analyzed in the report had already been in large part shared confidentially with public health agencies around the

world [including the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention]. The debate as to which data should be immediately made public after collection is long-running and complex. It is all too common for data from public health agencies to be used in scientific publications, with no recognition given to the teams involved in its collection. Whether the best means of recognizing the contribution of agencies is to have named representative authors from those agencies or corporate authorship of the whole organization is unclear. Overall, however, we emphasize that the contribution of WHO and the Mexican Ministry of Health to our Report was motivated by public health relevance, not authorship issues.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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PALEONTOLOGY

Much Hype and Many Errors

Richard F. Kay

Provocative and entertaining, *The Link* tells the story of a remarkably preserved fossil primate named *Darwinius masillae* and nicknamed “Ida.” The account is related by natural history writer Colin Tudge with Josh Young (who also coauthored *Pure Imagination: The Making of Willy Wonka and the Chocolate Factory*). Tudge and Young describe how Ida became entombed in an ancient lakebed and how it came to light 47 million years later. Apparently unearthed in 1983 near Messel, Germany, the specimen was cleaved in two and each part sold separately. The first part (now called Slab B) went to a Wyoming collector and was illustrated in 1994 (1). The second, more complete mirror-image half (Slab A) recently was sold for a stunning \$750,000 to the University of Oslo’s Natural History Museum, a deal brokered by paleontologist Jørn H. Hurum. Hurum then assembled what he refers to in the book as a “dream team” of other scientists to describe the reunited fossil (2). The specimen is claimed to be the most complete fossil primate ever found—and that is true at least in two dimensions, for it is crushed nearly flat. If we take the third dimension into consideration, other fossil primate skeletons, including some of *Darwinius*’s North American adapoid relatives, are as well or better preserved and have been in the literature since the 1920s (3).

As the title suggests, the authors claim that *Darwinius* is our ancestor and represents a linking form between lemurlike primates and anthropoids (the group to which monkeys, apes, and humans belong). As quoted by Tudge, paleontologists Jens Franzen and Philip Gingerich, two authors of the scientific paper (2), liken Ida’s impact on the world of paleontology to the equivalent of an “asteroid falling down to Earth” (Franzen) and a “Rosetta Stone” for primate evolution (Gingerich).

Indeed, Ida has had the public impact of an asteroid. The debut of *Darwinius* was a media juggernaut: the scientific paper, a public unveil-

ing by New York City’s Mayor Bloomberg, a Web site (4), coverage in *People* magazine, a television special, and the book all appeared within a week. On the day of the announcement, the traffic at the Web site exceeded that for www.sciencemag.org (5). But has this fossil rocked our understanding of primate evolution?

Of the book’s nine chapters, the first three and final are written by Young in a sensational style: “Hurum pulled out one final key card and opened the door... Geffen was stunned.” These parts are the most entertaining or, depending on one’s point of view, disturbing. Ida is a reprise of the seamier side of paleontology: the exploitation, with the complicity of major museums, of our natural heritage for profit. The rising commercial value of fossils in recent years (first “*Tyrannosaurus Sue*”) has dampened scientists’ efforts to find more and better fossils. For the record, most paleontologists obtain their specimens by groveling around on the ground, not by purchasing them at fossil fairs.



The Link Uncovering Our Earliest Ancestor

by Colin Tudge,
with Josh Young

Little, Brown, New York, 2009.
304 pp. \$25.99, C\$28.99.
ISBN 9780316070089.

Four of the chapters written by Tudge provide the natural historical context for the fossil. He reviews how the Messel coals formed in an anoxic lake that periodically farted CO₂, killing the teeming life that inhabited its waters and shores. He provides an accessible, although inaccurate, discussion of the causes and consequences of Eocene greenhouse climates. He attributes much of the decline of worldwide temperatures at

the end of the Eocene to the massive death of aquatic ferns in the Arctic and changing oceanic circulation attendant on the collision between India and southern Asia. But the timing is wrong: those two events occurred some 15 and 20 million years before the onset of our modern “icehouse” world at about 34 million years ago (Ma) (6, 7).

Tudge also offers a tour of primate ecology, behavior, and evolution that is often inaccurate and at times misleading. (For example, he misstates the age of the earliest New World monkey by 6 million years and that of the African anthropoid fossil *Apidium* by 10 million years.) Why are there so many errors? Perhaps because the book had no scientific editor to check facts or demand reviews that would have uncovered these inaccuracies. The book was rushed out, and the whole project from purchase of the fossil up to the media blitz was cloaked in secrecy.

In the chapter “Who and what is Ida?” we get to the heart of the book: where does *Darwinius* fit on the primate family tree? It belongs to an extinct Eocene primate group, Adapoidea (also called Adapiformes). Tudge reports: “Ida reinforces Gingerich’s idea of primate evolution that anthropoids descended from adapiforms. ... tarsiers, so long thought to be the closest living relatives of anthropoids, now are thought to belong on a quite different branch; and so are omomyids, which are clearly related to the tarsiers.”

This is the lemur-anthropoid theory, according to which living lemurs and anthropoids arose in the Eocene from different adapoid lineages whereas living tarsiers are a side group that arose from another Eocene group, the omomyids (8). Insofar as the theory posits an adapoid origin for lemurs and lorises, it is unobjectionable. There is evidence that adapoids are stem members of the lemur-loris radiation (9). The theory cannot be true, however, if tarsiers are more closely related to anthropoids than either is to lemurs. And the evidence for a tarsier-anthropoid clade is very strong. Long-known anatomical and develop-

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mental evidence has been convincingly reinforced by DNA sequence data and the sharing of SINEs, short segments of DNA inserted into the genome at apparently random positions (10–14). Support for a lemur-loris clade is equally strong; it forms a side branch to the tarsier-anthropoid group (10, 11, 13, 14).

In short, the interpretation favored by Gingerich and described by Tudge is incompatible with a basal split of anthropoids from lemurs and lorises. Adapoids cannot simultaneously be the source of lemurs, lorises, and anthropoids to the exclusion of tarsiers because tarsiers and anthropoids form a clade. So *Darwinius* cannot be both an adapoid and a monkey ancestor.

As to the search for our ancestors, a growing body of evidence suggests that the earliest anthropoids belong to the Eosimiidae, another Eocene group that was contemporaneous with adapoids. Eosimiids are known from jaws, teeth, a partial skull, and limb bones. They appear in Asia as early as 54 Ma and may have some African members (15, 16).

So, *Link*'s premise, that *Ida* is our ancestor, is fallacious. *Ida* is a lemur. While the search for anthropoid origins goes on, we shouldn't look for human ancestors in *Darwinius* or its close relatives. Interested readers who want to become better informed about early primate evolution should turn to K. C. Beard's *The Hunt for the Dawn Monkey* (17), an accessible, authoritative alternative.

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BROWSINGS

Summer World: A Season of Bounty. Bernd Heinrich. Ecco (HarperCollins), New York, 2009. 277 pp. \$26.99. ISBN 9780060742171.

This enticing account draws on two summers Heinrich spent “actively observant” at his rural Vermont home and his cabin in the Maine woods. He weaves the narrative of ecology, behavior, and physiology from his own sightings and simple experiments, working in research findings from the primary literature. The book follows the arc of the season from the first nodding heads of snowdrops through to the last autumnal cheeps and chirps of spring peepers and wood frogs. Although a few parts address the challenges posed by higher temperatures and limited water, Heinrich focuses on interactions among animals and plants—especially strategies used in feeding or reproduction. He supplements the often lyrical discussions with his own sketches and paintings, such as the illustration (above) of the tachinid *Belvosia bifasciata*, a large, striking fly that parasitizes silk-moth caterpillars.



RISK ANALYSIS

Five “Easy” Questions

Garry D. Brewer

Risk assessment is a commendable collection of efforts by varied scientists and numerous technologists to make complex human choices understandable. *Science and Decisions* acknowledges this even as it begins: “Virtually every aspect of life involves risk. How we deal with risk depends largely on how well we understand it.” Most of the book focuses on the theories, methods, and practices of risk assessment in public health, specifically as these evolved through the years at the U.S. Environmental Protection Agency (EPA). This National Research Council (NRC) report was commissioned by EPA and prepared by a special committee chaired by Thomas A. Burke (School of Public Health, Johns Hopkins University) working with the NRC’s Board on Environmental Studies and Toxicology.

Encyclopedic, *Science and Decisions* will be a standard reference and textbook for many years to come. Indeed, it may become this decade’s equivalent to the path-breaking

Science and Decisions
Advancing Risk Assessment
by the National Research Council.
Committee on Improving Risk Analysis Approaches Used by the U.S. EPA
National Academies Press, Washington, DC, 2009. 421 pp. Paper, \$54.95.
ISBN 9780309120463. www.nap.edu/catalog.php?record_id=12209

“Red Book” of 1983, *Risk Assessment in the Federal Government* (1), and to three following milestones from the 1990s (2–4). Well organized and carefully written, *Science and Decisions* leaves scarcely a technical risk assessment topic uncovered within its 421 pages. (There is no index, alas.)

The history, practice, and current state of risk assessment are covered succinctly in the opening three chapters. For anyone new to the field, this is essential reading. For everyone else, these chapters remind one of a quarter century of accomplishments and highlight numerous challenges remaining. These challenges are identified and considered carefully, especially in four chapters that deal with human, institutional, and even “irrational” aspects of risk assessment.

One chapter is devoted to uncertainty and variability. These are explored along with many other concepts and practices of technical risk assessment so as to expose lingering problems—most of which are decidedly non-technical: “The question is not often about better ways to do these analyses, but about developing a better understanding of when to do these analyses.” The important practice of

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cumulative risk assessment merits a chapter and also results in a list of questions, the answers to which should improve decision-making. Again, the questions are decidedly nontechnical: “[W]e recognize that the topic of cumulative risk assessment raises important questions about the bounds between risk assessment and other lines of evidence that may inform risk-related decisions.” The concluding two chapters on improving the utility of risk assessment and improving risk-based decision-making aim to go beyond the “Red Book” and provide a map and guide to do so.

The report is a major accomplishment. It courageously addresses questions that many of its legendary predecessors and most other works on risk assessment either don’t ask or fail to answer. These lapses occur because the rational expectations and technical strengths of risk assessment overshadow the human, often “irrational” and emotional, complications that are manifest in and define risk perception. These distinctions are not trivial, for as one discovers repeatedly, perceptions are the reality decision-makers must confront and manage. From the decision-maker’s perspective, this fundamental issue can be addressed by raising and trying to answer five “easy” questions:

What’s the problem? Although simply posed, this question can be answered only with great difficulty. Problems in which risk figures prominently are socially constructed and so depend on diverse perceptions mediated through numerous sets of human values. Only in very special, constrained situations will they be “well defined” and thus amenable to clear specification and possible solution. Many success stories in risk assessment in fact developed from special and constrained problem definitions. In more usual circumstances, problems are “wicked” and have no definite formulation and no clear point at which they are solved (5, 6). Problem definition is therefore ongoing because in practice many different versions of it will exist. It is also crucial because examination of these differences will inevitably result in dynamic responses to the question of “what’s the problem?” and thus affect one’s capacity to decide and manage.

For whom is something a problem? “The problem” is not simply what a study’s sponsor or consultant’s client wants it to be, nor is it limited to what different scientists think,



We already know what to do. Risk assessment is not needed in cases such as coal-fired power plants, where the environmental impacts are known.

know, and state it to be. It is equally what those implicated in it imagine it to be. In the public health world of *Science and Decisions*, an important answer is that different kinds of human beings will respond differently to the identical exposures or doses. Infants get cancer more readily from the same carcinogenic agent and dose than healthy young adults do. In the more commonplace world, the answer is even more complicated.

The more-than-two-decade struggle to deal with America’s nuclear power plant waste at Yucca Mountain, Nevada, is illustrative. Here the answer is more about current fears and lack of trust for many in the general public than it is about rational calculations of leaking waste storage canisters for engineers or radiation exposure for health specialists thousands of years in the future. Around \$10 billion worth of scientific study, arguably the world’s most ambitious risk assessment, has so far been stymied by human psychology and public opposition (7, 8).

Who is the decision-maker? Blithely postulating the existence of some powerful person who “makes decisions” and then not figuring out who that might be is commonplace. *Science and Decisions* confronts this issue—“Historically ... guidance arising out of National Research Council risk-assessment reports has been directed mainly to improving agency risk assessments [not] to future decision-making”—and then speculates on numerous possibilities for who the decision-maker might be. That is a good start and should be routine.

Why do risk analysis if we already know what to do? People should not smoke, overeat, engage in risky sex, or drink excessively. Heart disease, lung disease, diabetes, AIDS, and other ways to die all happen earlier and more frequently if people do. There is no par-

ticular need to carry out a thorough risk assessment to know what to do to reduce and delay deadly consequences of these behaviors (9). Likewise, effluents from chicken-, pig-, and cattle-feed lots increase nutrient loads on ecosystems that in turn stimulate algal blooms, hypoxia, degraded habitats, increased chemical loads on humans downstream, and economic losses to society far exceeding profits received by industrial food producers. A similarly evident situation exists with coal-fired power plants and all their harmful consequences for humans and

ecosystems. No risk assessment is needed to tell us what to do in such cases. These problems are human, emotional, intentional, and political—not analytical.

So what? Ultimately, one needs to ask, are we any better off because of risk assessments? Fearful thinking by the public about getting fatal cancer is a bedrock justification, usually unstated, motivating much traditional risk assessment. Because everyone eventually dies of something, has all the time, trouble, and expenditure been worthwhile? Or is most of what passes for risk assessment primarily pursuing spurious rigor, using increasingly incomprehensible (to the public and to “decision-makers”) techniques, in an effort to limit discussion and to control decision-making?

The five easy questions are truly wickedly hard to answer. Amazingly, courageously, and encouragingly, these questions and the possibilities they invoke are raised and discussed in *Science and Decisions*.

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SCIENCE EDUCATION

Introducing Modern Science into Schools

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Young people in Europe are becoming increasingly disinterested in science at school and are moving away from studying science at university, making it difficult to recruit the engineers and scientists needed to support technology-based economies (1). Several aspects of contemporary science teaching appear to be discouraging students: The pedagogy lacks variety, the quality of teaching is less engaging than for other subjects, and the content often appeals preferentially to male students (2–4). Most pedagogy is heavily reliant on transmission of facts and often lacks epistemological learning goals, which seek to offer an understanding of how we know that scientific facts are true. Many current teaching methods and curricula are designed to prepare students to pass tests by rote learning, but fail to develop cognitive skills needed in science (5). Coursework investigations are also widely criticized for being rather artificial exercises, which do not reflect the true nature of science as a process of inquiry (6).

Scientific inquiry—mimicking the scientific learning process in the classroom—is a recent approach to improve the way science is taught in school (7). Exposing students to learning processes that closely resemble scientific discovery is a way of enhancing their scientific literacy. But a classroom is not a research laboratory. The huge costs of scientific equipment, together with additional restrictions imposed by health and safety regulations, severely limit the possibilities of conducting molecular biology experiments in school labs.

Herein lies the biggest challenge—to present sophisticated technologies behind today's science in a school setting (8). The only way to ensure that all students have an insight into new and important fields, especially in biology, is to include these in curricula and examinations (9). However, this can only be achieved in parallel with regular training of teachers to teach the new content (3, 7, 9).

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Many teachers have not worked as researchers and find it hard to understand the methodology of science (9). Moreover, they are often hesitant to teach subjects in which they are not expert. If they set up predictable experiments, with expected results, it may impair the development of students' analytical skills (8). But most educational systems have not developed effective mechanisms to keep teachers up to date with contemporary science (6), which leaves teachers with limited access to continuing professional development (CPD).

ELLS at EMBL

The European Molecular Biology Laboratory (EMBL), an intergovernmental research organization funded by public money from 20 member states, includes in its mission an active education effort. The European Learning Laboratory for the Life Sciences (ELLS) is EMBL's link between research and schools (10).

ELLS provides CPD for European high-school teachers, based on state-of-the-art molecular biology research at EMBL, in an approach that combines formal teaching and exercises with informal discussions. Since its inception in 2003, over 700 teachers from 22 countries have participated in ELLS CPD programs. The main aim of ELLS is to empower teachers to develop innovative teaching material and strategies, often in parallel with EMBL scientists who are closely involved in the design of the resources. ELLS updates teachers' knowledge about new scientific concepts and methods that are appearing on curricula and provide a special intellectual challenge to teachers and students alike. By targeting teachers, ELLS aims to support a long-term, bottom-up change in the way school science is taught.

Science curricula in Europe are generated at the national level, and changes in curricula content and teaching standards are often driven by the results and recommendations of national or international surveys and expert reports (7, 11, 12). Several institutions in Europe are addressing the challenges of bringing modern science into schools [see

A multinational research institution provides advanced training to help high-school teachers bring inquiry into the classroom.



The Virtual Microarray Game. Students play the virtual DNA microarray game in the classroom. The game consists of a custom-made mat, Velcro, and flashlights and guides students step by step through microarray technology.

supporting online material (SOM) for a list of initiatives and educational resources]. Unlike national CPD programs, ELLS's scope is focused on rethinking, redesigning, and improving resources for high-school science, without directly affecting national science curricula. As a result of this status, the facility occupies a unique, impartial position in science education in Europe.

Mimicking Scientific Research in School

Recognizing the importance of hands-on experimentation to enhance students' scientific literacy, ELLS places lab-based activities at the center of its CPD courses (LearningLABs).

The facility regularly hosts small international groups of high-school teachers to work alongside EMBL's scientists, building teachers' confidence and expertise to conduct such wet-lab activities at school. ELLS's inquiry-based modules require relatively simple lab materials, and the facility also lends equipment to local schools.

ELLS has developed scientific games that simulate the steps of modern molecular biology techniques such as sequencing and microarrays, which have quickly found their way into curricula. For example, the Virtual DNA Microarray game follows the steps that researchers take in performing and analyzing microarray experiments, which capture the expression of millions of genes simultaneously, the game elucidates basic microarray concepts and the multiple applications of this technology (13) (see the figure).

Teachers also complement their "researcher" experience at ELLS by attending seminars and visiting EMBL's research facilities. In addition, they have access to primary research literature, as well as versions modified for use in classrooms to develop critical thinking (4) and to stimulate students to ask questions and to engage higher-level cognitive skills (14). Reading primary literature also introduces the peer-review process, underlining the collective aspect of the scientific enterprise.

Blended Learning Approach

Research on how to introduce new technologies in the classroom indicates the importance of connecting concepts to concrete examples in an interdisciplinary way (6). ELLS's educational resources incorporate knowledge from different scientific fields, combining wet-lab and online learning elements, and are specially designed to illustrate real-life applications of science and technology. For example, LearningLAB participants assume the role of developmental biologists, employing classical molecular biology technology to screen wild-type and eyeless mutant embryos of medaka fish. They make use of bioinformatics tools to search for orthologous genes responsible for similar eye phenotypes in other organisms, discovering for themselves links between gene evolution and phylogenetics.

The impact of science on society is also an important contextual link that can capture students' interest (6). Role-plays and other games (13–16) are used to engage participants in discussions on topics such as genetic testing. A webcast lecture series embeds research in a more general context. Presented by high-profile women scientists, the web-

casts target students—especially girls, teachers, and the general public (17).

Resources, Development, and Evaluation

ELLS's education resources aim to update and complement relevant thematic areas common to national high-school science curricula. EMBL scientists and ELLS's staff are in a unique position to sense which high-impact discoveries are likely to become incorporated into national school curricula. Collaborating with teachers and scientists, ELLS develops resource content and format to better convey cutting-edge science to school settings. Resources are first developed in prototypes, which undergo rigorous testing in LearningLABS, science festivals, and school lessons. They are then refined on the basis of user feedback and are finally produced in several languages and made freely available through the ELLS TeachingBASE (18).

Areas of modern molecular biology such as systems biology, bioinformatics, molecular evolution, and molecular medicine are included in high-school curricula, but teaching resources are limited. In this sense, ELLS can provide valuable resources for teachers. From surveys, we know that teachers experience ELLS LearningLABS very positively and are able to incorporate activities into classroom teaching (figs. S1 and S2).

iNEXT

EMBL has recently launched the interactive Network for Experimental Training (iNEXT) in which science education researchers and EMBL scientists assess inquiry-based approaches and materials, not only to evaluate their impact on teachers' competency and confidence, but especially to assess their effectiveness in helping students understand and retain scientific concepts (19) (fig. S3). iNEXT is intended to provide long-term practical support for teachers and students, allowing them to work alongside scientists in the development of prototype, inquiry-based teaching modules. Teachers become knowledge experts and develop a suitable pedagogical format for the activity, while the scientists ensure the soundness of the scientific content. Prototype modules will cover new concepts in evolution, developmental biology, bioinformatics, and the emerging field of metagenomics. iNEXT offers teachers the possibility of implementing ideas on novel instructional approaches and should also greatly enhance the acceptance of the materials by their colleagues.

iNEXT draws on an editorial board composed of scientists from different disciplines, teachers, and education experts who influence the creation and updating of mate-

rials. A Web forum facilitates this process.

Reshaping teaching and generating change in science education is a long-term effort. We do not aim to prescribe a standardized European life sciences curriculum, but rather to develop a set of tools for European high-school teachers to enhance their own school environment and to encourage students to understand the complexity and uncertainty of scientific endeavor.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5944/1077/DC1
SOM Text
Figs. S1 to S2

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PALEONTOLOGY

Flourishing After the End-Permian Mass Extinction

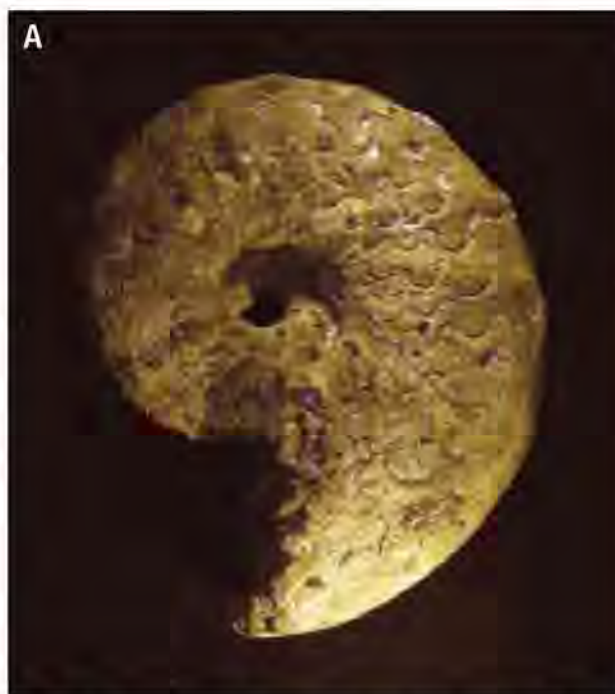
Charles R. Marshall¹ and David K. Jacobs²

Two hundred and fifty-two million years ago, the Paleozoic Era came to a cataclysmic close with the end-Permian mass extinction, when as much as 85% of readily fossilizable marine species became extinct. It took 5 million years for the biosphere to begin to recover from the event. At least this has been the conventional view. However, on page 1118 of this issue, Brayard *et al.* (1) show that ceratitid ammonoids (see the figure, panel A) recovered much faster than did most other marine groups, attaining considerable diversity just 1 million years after the mass extinction. Moreover, these mollusks reached a peak in their diversity at the end of the Early Triassic, when the diversity and body size of most other groups (particularly bivalves and gastropods) was still depressed (2). What do these data tell us about the post-apocalyptic world of the Early Triassic, and about the cause of the end-Permian extinction event itself?

The cause of the end-Permian mass extinction has long been controversial (3). There is increasing agreement that toxic waters decimated bottom communities in shallow waters, but it remains unclear whether the kill mechanism was hypercapnia (high CO₂ levels) (4), euxinia (anoxic water infused with H₂S) (5), or something else. There is even less agreement on what might have caused the toxicity.

One possibility is that the Siberian traps were responsible—a vast outpouring of basalts that released huge volumes of CO₂ as they passed through thick coal sequences. Between 1 and 3 million km³ may have been disgorged (enough to bury Texas under 1.4 to 4.3 km of lava). Under this scenario, the increased CO₂—with atmospheric concentrations possibly 10 times as high as at present—led to massive global warming. This warming decreased the oxygen content of the oceans, permitting the biologically mediated generation of H₂S in the deep oceans, which, upon upwelling, caused the extinctions (6).

Whatever the ultimate cause(s) of the



Tips for flourishing after a mass extinction. *Ceratites nodosus* (MCZ-7232) (A), from the Triassic of Germany, was similar to the ceratitid ammonoid species that thrived in the water column in the Early Triassic (1), while bottom-dwelling species languished. Key to the ceratitids' rapid success after the end-Permian mass extinction were their ecological tolerances, which may be inferred by reference to their closest living relatives, the coleoids (squids, octopuses, and cuttlefish), including the low-oxygen specialist *Vampyroteuthis infernalis* (B).

extinction, the proximal cause appears to have been the inability of many species to handle the physiological demands of a changed ocean chemistry. Evidence that conditions remained difficult for 5 million years after the extinctions comes mainly from the observation that the diversity and size of fossil bivalves and gastropods remained low, indicating stressed conditions (2). Furthermore, the carbon cycle was unusually volatile (2), although the exact meaning of this volatility is not understood (7). Another indication of difficult times is the delay in the recovery of reef communities: The two major clades of Paleozoic coral (the Rugosa and Tabulata) became extinct at the end of the Permian, but modern corals (the Scleractinia) did not emerge until the Middle Triassic (8).

The ammonoid data reported by Brayard *et al.* suggest a much more rapid recovery, at least for part of the biosphere. Unlike the bottom-dwelling gastropods and bivalves, ammonoids live in the water column. Thus, Brayard *et al.*'s study suggests that conditions in the water column were better than those on the bottom. Or does it?

If the ceratitid ammonoids were active predators requiring high oxygen, this would indeed suggest a healthy water column, but if they preferred low oxygen conditions, then

their rapid rise in diversity suggests that the water column may also have been stressed, like the shallow bottom waters in the Early Triassic. The latter scenario is consistent with a major extinction of ceratitids at the start of the Middle Triassic (1), when the bottom dwellers began their full recovery. In fact, the ammonoids are a characteristic component of the lost biotope (9)—faunas dominated by taxa that thrived in the water column in the Paleozoic and Mesozoic while the benthic fauna was low in diversity. Furthermore, ammonoids are known to have diversified quickly after a wide range of environmental crises, not just after the end-Permian mass extinction.

To better understand the meaning of Brayard *et al.*'s data, we need to know more about the biology and physiological tolerances of ammonoids in general, and of ceratitids in particular. Although the group is extinct, we can turn to their nearest living relatives, the coleoids (squids, octopods, and cuttlefishes)—and also *Nautilus*, which looks superficially similar to the ammonoids (10). Although many modern coleoids are active predators in oxygenated waters, others like *Vampyroteuthis infernalis* (“vampire squid from hell”) (see the figure, panel B) and *Nautilus* require little oxygen. These species lie deep in the evolutionary trees of living coleoids and liv-

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ing cephalopods, respectively, suggesting that a tolerance for low oxygen was ancestral for living cephalopods. These observations support an affinity for low-oxygen conditions for many ammonoids, consistent with the dysoxic paleoenvironmental setting in which they are frequently found.

Identification of physiological and ecological commonalities among both the victims and the survivors (4) is a powerful route to elucidate the causes of major biotic turnovers, including the cataclysmic end-Permian mass extinction

(3). The task of establishing the environmental requirements of extinct fossil groups can be aided by studying their living relatives. These data will allow increasingly refined interpretation of past environments, including those associated with mass extinctions.

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PHYSICS

Chasing Arcs in Cuprate Superconductors

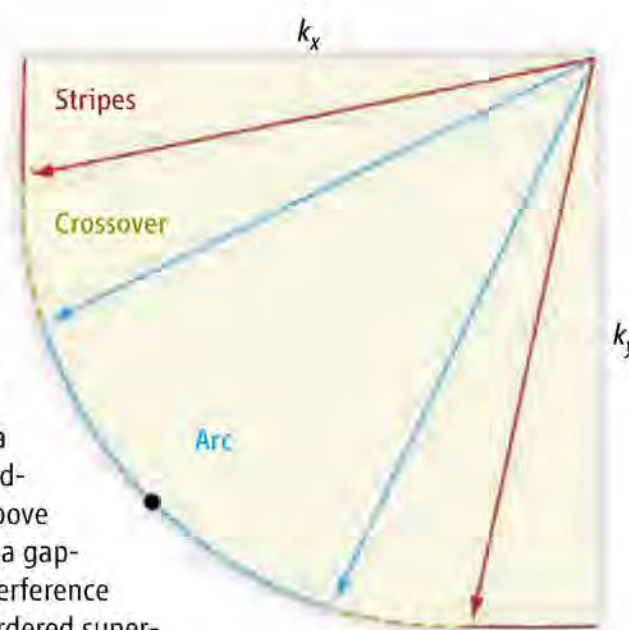
Michael R. Norman

Important clues to the origin of high-temperature superconductivity in cuprate compounds lie within the normal phase of these compounds, which forms above the transition temperature T_c . One unusual feature of the normal phase is the presence of a pseudogap; depending on the momentum of the charge carrier, its excitation energy is either zero or finite (1). Two reports in *Science*, by Pushp *et al.* (2) in June and by Lee *et al.* (3) on page 1099 of this issue, have used scanning tunneling microscopy to provide dramatic new insights into the pseudogap phase and to elucidate how the electronic excitations, both above and below T_c , differ for different values of the carrier momentum.

The cuprate superconductors are formed by chemically doping compounds that simple energy-band models predict to be conductors, but that actually are insulators because of strong electronic correlations. Chemical doping creates charge carriers and, at some optimal level, maximizes T_c . For lower doping levels, the “underdoped” normal state is characterized by a pseudogap—that is, a partial depletion of the charge carriers (1). Angle-resolved photoemission studies, which probe electronic states in momentum space, were the first to show that some parts of the Fermi surface that separates occupied and unoccupied states are gapless as in a conventional metal, but other parts have an energy gap. That is, the Fermi surface of the underdoped normal phase is truncated (4, 5) when portrayed in momentum space, forming arcs instead of full curves that bound a region (see the figure).

The three-part electronic structure of underdoped cuprates. The curve represents the Fermi surface in one quadrant of momentum space, defined by momenta in the x and y directions of the copper oxide planes, k_x and k_y . In the superconducting state at temperatures below T_c , the lower-energy blue region has a simple d-wave gap; the node, where the energy gap vanishes, is marked by a black dot. In the antinodal regions marked in red, the energy gaps are largest, and there are incoherent states with a stripe-like pattern. The dashed green region marks a crossover between these two regimes, as seen in the studies of Pushp *et al.* and Lee *et al.* In the normal state above T_c , the energy gap in the blue region collapses to form a gapless arc. In the dashed green region, quasiparticle interference patterns remain that imply the presence of phase-disordered superconductivity. The incoherent red regions are largely unaffected by T_c .

Scanning tunneling microscopy studies of cuprate superconductors clarify the origin of their unusual electronic structure.



These arcs have provoked controversy ever since they were first observed, because Fermi surfaces should form closed contours (which in special circumstances can reduce to gapless points). The idea of a Fermi surface holds rigorously only at absolute zero temperature; thus, the arcs may be generated by thermally broadening the nodes (the gapless points) of the d-wave superconducting state into segments. It is also possible that they are only part of the boundary of a closed region, or pocket, but that the rest of the boundary is too weak to be observed.

Interest in Fermi arcs has been renewed by a number of recent studies that tie them into the debate over the mechanism of superconductivity. The arc length appears to scale linearly with temperature (6), which supports the idea that they originate from thermally broadened d-wave nodes. However, recent quantum oscillation studies (7, 8) have found evidence for closed pockets in the under-

doped regime; these pockets are likely to be a result of some ordering of the charge carriers. Some recent photoemission studies also show evidence for pockets (9–11).

Scanning tunneling microscopy also provides information on the local density of electronic states as a function of energy, and although the data are obtained in position space, they can be mathematically inverted to provide a momentum-space picture. This technique is complementary to photoemission in that it can reveal local variations in the material. Use of this technique has revealed that the electronic excitations of the superconducting phase are inhomogeneous in real space (12). For underdoped samples, this inhomogeneity is so drastic that a localized stripe phase forms that dominates the electronic structure on an energy scale comparable to the pseudogap (13).

However, lower-energy excitations appear to be quite homogeneous. Pushp *et*

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al. show that below T_c , these low-energy excitations exhibit a simple d-wave gap as observed by photoemission. Using different analyses, both Pushp *et al.* and Lee *et al.* conclude that, at a certain momentum and energy, these excitations characteristic of the superconducting phase cross over to those of the stripe-like states. Lee *et al.* find that both in the d-wave gap region and in the crossover region, the states are phase-coherent in that they support quasiparticle-like interference patterns. Pushp *et al.* speculate that T_c is proportional to the size of the region of momentum space that supports this simple d-wave gap.

Both groups find that the node of the d-wave gap opens up into an arc in the normal state, just as seen in photoemission, although Pushp *et al.* infer the presence of a node below T_c while Lee *et al.* contend that the arc is still present. More interestingly, the latter find that the states in the crossover region above T_c continue to support the same quasiparticle-like interference pattern as seen in the superconducting state. The implication is that the pseudogap phase, at least for a range of temperatures above T_c , is a phase-disordered version of the d-wave superconducting state, and both groups agree that the arc is indeed a thermally broadened node.

What are the implications of these latest findings? An unusual gap anisotropy is

observed, in which a simple d-wave gap near the nodes crosses over to a much steeper angular dependence away from the nodes. This functional form is reminiscent of the “two-gap” behavior recently inferred from a number of photoemission studies, as well as other spectroscopies such as Raman scattering (14). This two-gap behavior can be explained as either a distortion of the simple d-wave gap caused by the presence of higher harmonics, or by the addition of a completely different energy gap that is connected with some other ordering tendency besides superconductivity. However, Lee *et al.* argue that at least the states in the crossover region are still characterized by a phase-incoherent superconducting gap, given the persistence of the same interference pattern above T_c and the disappearance of certain peaks in the Fourier transform that can be related to the phase of the d-wave order parameter.

However, in the “antinodal” region beyond the crossover region, where the d-wave superconducting gap should be largest, something different is going on. The stripe-like pattern that characterizes these higher-energy states does not at all resemble what is expected if the gap in this region of momentum space is caused by superconductivity. Rather, Lee *et al.* find that it resembles a glass-like state of localized bonds (13). These bonds may

represent spin singlets, and it could well be that d-wave superconductivity is a low-energy manifestation of these singlets. These bonds could also represent a quasi-ordered charge-density and/or spin-density wave phase that competes with superconductivity. As the doping level increases, this phase is suppressed, and Pushp *et al.* find that this effect leads to an expansion of the region of momentum space that can support superconductivity. Which of these pictures, competing order versus preformed singlets, is correct will have much to tell us about the origin of high-temperature superconductivity.

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CELL BIOLOGY

Using Taste to Clear the Air(ways)

Sue C. Kinnamon¹ and Susan D. Reynolds²

Epithelial cells that line the human airway are constantly bombarded by environmental hazards, including toxins, irritants, viruses, and bacteria. The airway rids itself of these agents by secreting mucus to “capture” harmful substances and increasing the beat frequency of motile cilia on epithelial cells to sweep the mucus out of the system. Protective reflexes such as coughing are also initiated. The mechanisms used to detect and respond to harmful agents are poorly understood. On page 1131 of this issue, Shah *et al.* (1) report that cultured human airway epithelial cells use elements of the bitter taste cellular signaling pathway to detect and eliminate potential noxious agents from the airways.

Surprisingly, Shah *et al.* find that several signaling molecules that respond to bitter compounds are expressed by ciliated airway epithelial cells, including the T2R family of bitter taste receptors, the heterotrimeric GTP-binding protein (G protein) α -gustducin, and the enzyme phospholipase C- β 2 (PLC- β 2). T2R receptors and G proteins are located in discrete zones on the ciliary shaft, whereas PLC- β 2 is located just beneath the cilia in the apical portion of the cell. When stimulated with bitter compounds, the ciliated cells respond with an increase in intracellular calcium ion (Ca^{2+}) concentration and a concomitant increase in ciliary beat frequency, which in vivo would presumably aid in removing noxious substances from the airways.

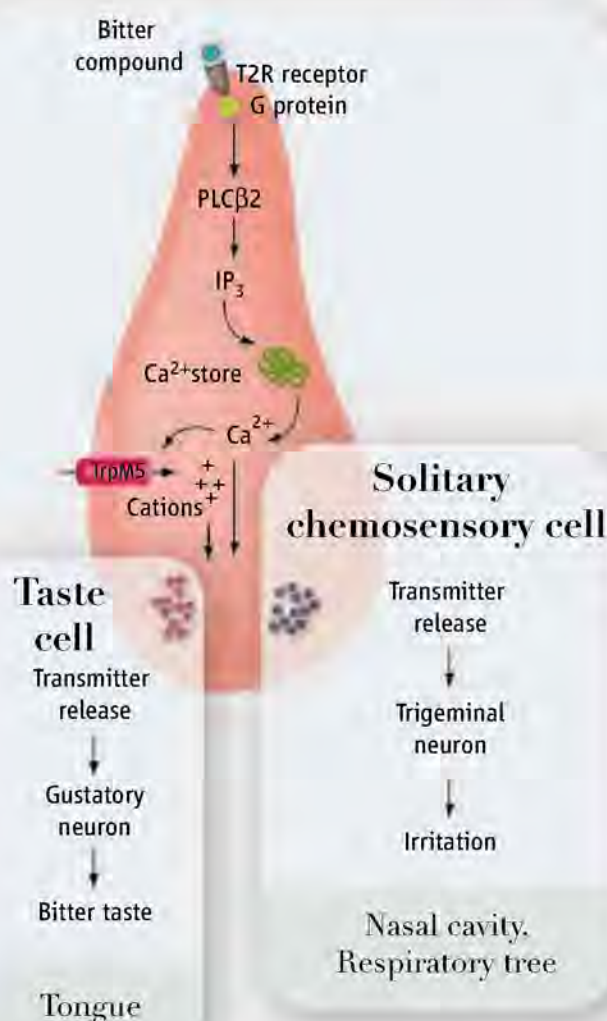
The study by Shah *et al.* is the first report of sensory function in motile cilia. It has been assumed that sensory receptors are only expressed on primary cilia, and that

Cilia of epithelial cells in the human airway increase their beating frequency in response to compounds that are sensed through receptors for bitter compounds.

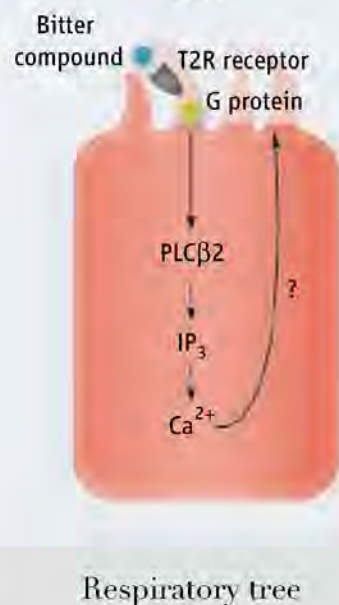
motile cilia have a purely motor function (2). However, in the green alga *Chlamydomonas reinhardtii*, ~25% of the proteins found in flagella—the tail-like projection used for locomotion—function in signaling, suggesting a role for motile cilia in detecting light, oxygen, redox state, and small ligands (3). T2R receptors were not detected in a recent proteomic screen of human airway cilia (4), however. This difference may be due to specialization of ciliated cells in subregions of the airway. Shah *et al.* used proximal airway epithelial cells, which respond to certain agonists by increasing their ciliary beat frequency (5, 6). By contrast, cilia on distal airway cells beat at maximal frequency and are refractory to agonists that increase intracellular Ca^{2+} concentration (7, 8). Thus, use of this in vitro culture system may have facilitated identification of a molecularly distinct ciliated cell subset.

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Bitter responses. A similar signaling pathway is initiated by bitter compounds in taste cells and solitary chemosensory cells. Activated T2R receptors trigger the production of inositol 1,4,5-trisphosphate (IP_3) and release of Ca^{2+} from internal stores. Ca^{2+} activates a nonselective cation channel, TrpM5, which depolarizes the cell and together with Ca^{2+} evokes the release of a transmitter that activates a target sensory neuron. In ciliated epithelial cells, bitter tastants signal through a similar pathway, but the functional outcome is an increased rate of ciliary beating.



Ciliated epithelial cell



Shah *et al.* demonstrate specific expression of T2R on cilia of cultured airway epithelial cells. However, immunohistochemical analysis detected other components of the T2R signaling pathway (α -gustducin and PLC- β 2) in secretory cells of human and rat airway tissue (9) and on human pulmonary neuroendocrine cells (10). These observations raise several points that will provide a focus for future studies. First, if T2R receptors are present in human airway cells in vivo, are they restricted to ciliated cells of the bronchial surface epithelium and/or gland ducts? Second, the secretory

cells that are presumably present in cultured bronchial airway cells may lack α -gustducin and PLC- β 2. Is this a consequence of cellular origin or in vitro differentiation? Also, is mucus secretion by secretory cells coupled to the increase in intracellular Ca^{2+} concentration that ciliated epithelial cells exhibit in response to bitter substances? If so, this coordination would facilitate clearing the airways of noxious agents.

Why should airway epithelial cells express bitter “taste” receptors? There are about 25 members of the T2R receptor family in humans, each responsive to a broad array of bitter compounds when expressed in heterologous cells (11). Bitter receptors respond to potential toxic substances in foods. But T2R receptors are present in tissues other than taste buds. In the gut, these receptors are expressed in some enteroendocrine cells. When stimulated, they release hormones that modulate gut motility or evoke reflexes controlled by the vagus nerve (12). T2R receptors are also expressed in solitary chemore-

ceptor cells of the nasal epithelium (13). These cells respond to a variety of irritants, including stimuli that would taste bitter. Activation of these cells evokes protective airway reflexes such as sneezing, coughing, or apnea (14). In all cases studied to date, activation of T2R receptors activates a similar cell signaling pathway that results in a protective reflex to rid the body of the noxious agent (see the figure). In an innervated cell, the increase in intracellular Ca^{2+} concentration activates a nonselective cation channel, TrpM5, which depolarizes the cell and evokes the release of transmitters that activate target sensory nerves. In ciliated epithelial cells, the increase in intracellular Ca^{2+} concentration evokes an increase in ciliary beat frequency. The molecular details of the latter response now need to be fleshed out.

What is the natural ligand for T2R-expressing cells in the airways? Whereas taste cells and enteroendocrine cells are likely to encounter ingested bitter toxins, most bitter compounds are not volatile, but could be encountered as fine particulates. Shah *et al.* suggest that airway epithelial cells may respond to products secreted by pathogenic bacteria. This may also be a natural stimulus for solitary chemoreceptor cells in the airways (15, 16). T2R receptors in vitro respond to lactones (17), which are chemically similar to quorum signaling molecules secreted by Gram-negative bacteria. It will be interesting to determine if the proximal airway cells respond to these compounds.

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CELL SIGNALING

Blocking Akt-ivity

David F. Restuccia and Brian A. Hemmings

Aberrations in cellular signaling pathways that involve the enzyme Akt (also called protein kinase B) are implicated in diverse diseases, including cancer, diabetes, and neurodegenerative disorders (1, 2). Thus, proteins involved in Akt activation and signaling are potential targets for therapeutic intervention. In fact, drugs directed against some of these targets are now in clinical trials for treating cancers, and the inhibition of Akt activation and signaling remains a major goal of drug discovery (3, 4). On page 1134 of this issue, Yang *et al.* (5) identify a chemical modification of Akt that controls its activation, identifying another potential means to inhibit this kinase in human cancers.

An important step in Akt activation is its translocation from the cytosol to the plasma membrane, where it becomes activated in response to the stimulation of growth factor receptors at the cell surface. However, the mechanisms that control this membrane localization are not clear. Akt possesses a PH domain, which binds to the molecule phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) in the plasma membrane. Similarly, the enzyme phosphoinositide-dependent protein kinase 1 (PDK-1), which phosphorylates and thereby activates Akt, localizes to the plasma membrane by binding to PIP₃. The membrane localization of PDK-1 triggers the recruitment of Akt to the membrane (6), though it is unclear precisely how. Now, Yang *et al.* show that this process is even more complicated (see the figure).

The authors identify Akt as a target of TRAF6, an E3 ubiquitin ligase. Ubiquitin ligases attach a small protein called ubiquitin to target proteins, which induces their degradation or promotes interactions with other proteins to transduce signals. These effects are distinguished by the attachment of single ubiquitin moieties to a protein substrate (monoubiquitination) or chains of ubiquitin proteins (polyubiquitination), as well as by the specific lysine residue that is modified. By ubiquitinating Akt, TRAF6 promotes Akt translocation to the plasma membrane, where it becomes phosphorylated. In cells lacking TRAF6, ubiquitination, membrane localization, activation, and signaling of Akt

were impaired in response to treatment with growth factors.

The amino acids modified by TRAF6 are lysine residues at positions 8 (K8) and 14 (K14), both of which are monoubiquitinated and lie in the PH domain. Mutation of either lysine residue to arginine impaired Akt activation. The K14R mutation specifically disrupts Akt interaction with PIP₃ (7). However, Yang *et al.* found that the K8R mutation did not affect binding to PIP₃. Nevertheless, membrane localization of this mutant was impaired in response to growth factors. Thus, by ubiquitinating Akt on two specific residues, TRAF6 promotes localization of the kinase to the plasma membrane for subsequent activation. Ubiquitination may cause a conformational change that enables Akt to interact with a protein that transports the kinase to the membrane. Ubiquitination of the protein neurotrophin receptor interacting factor (NRIF) by TRAF6 allows NRIF to associate with the protein p62. The resulting complex is then able to translocate to the nucleus (8).

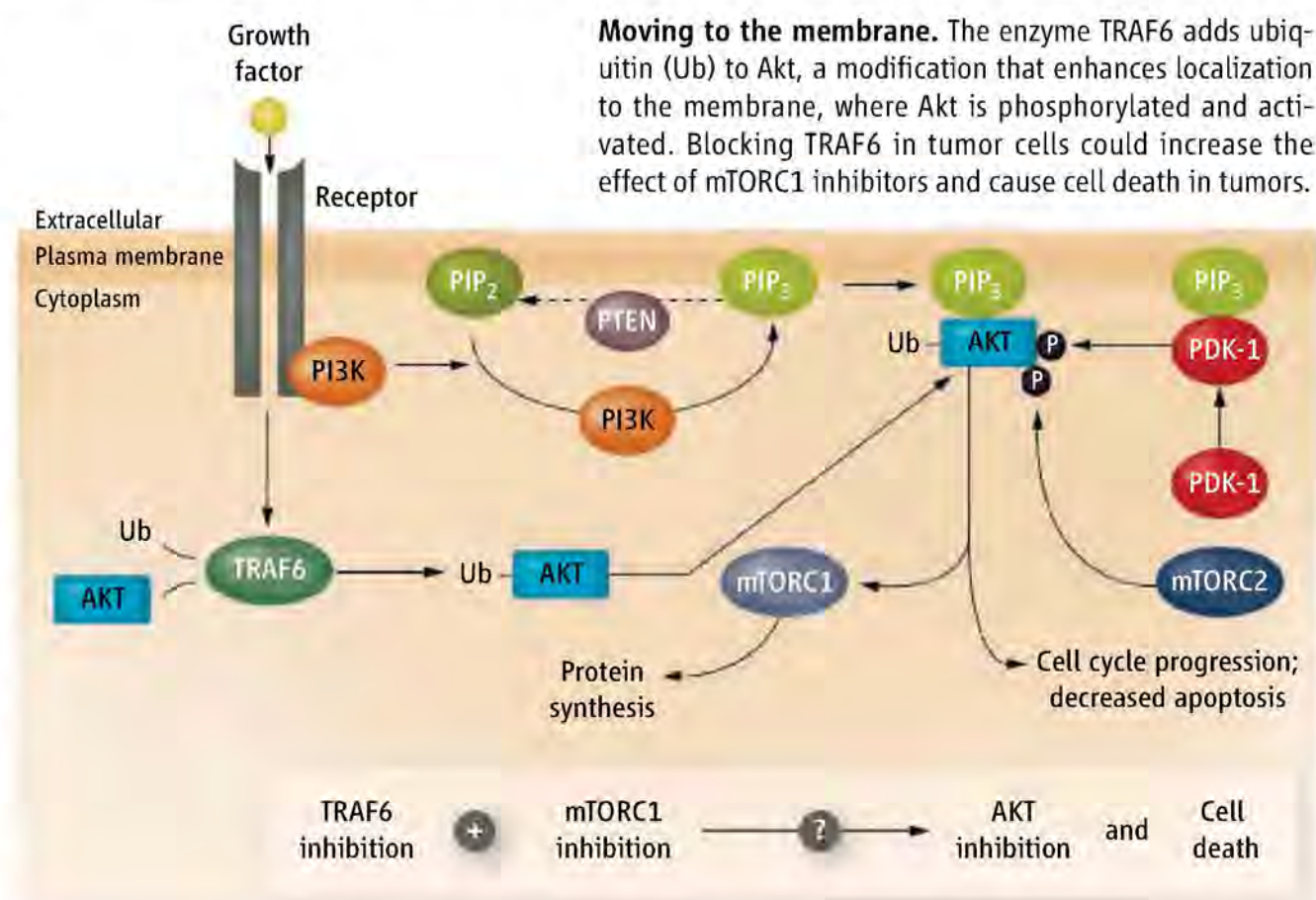
To determine whether TRAF6 is an effective target for inhibiting oncogenic Akt hyperactivation, Yang *et al.* examined an activated, mutant form of Akt identified in tumor samples of patients with breast, colorectal, or ovarian cancer (9). In this mutant, glutamic acid at position 17 is replaced with lysine (E17K), which increases interaction of its

Inhibiting the addition of ubiquitin molecules to the enzyme AKT could improve the effects of anticancer drugs.

PH domain through a conformational change with PIP₃. Enhanced membrane association of the mutant form of Akt increases its activation, even in the absence of growth factors. The E17K mutant also displayed greater overall ubiquitination—lysine residues at positions 8, 14, and 17 become modified—and its ubiquitination was further potentiated when TRAF6 was overexpressed in cells. Mutating the K8 residue in this mutant decreased Akt activation and downstream signaling. Thus, the Akt mutant uses ubiquitination to attain its hyperactive state.

The authors extended this concept by depleting TRAF6 (by RNA interference) from a human tumor cell line that expresses the hyperactive, mutant form of Akt, and then injecting these cells into “nude” mice (animals that do not mount an immune response to foreign cells). Tumor formation by these cells was severely impaired compared to tumor cells expressing TRAF6 that were injected into animals, consistent with the potential of TRAF6 inhibition to stop tumor growth.

Could inhibiting TRAF6 be an effective clinical therapy for human cancer? Although no inhibitors of TRAF6 are currently available, blocking the function of E3 ligases has shown effective antitumor properties in preclinical studies, and such inhibitors are moving toward clinical trials (10). Studies of the E3 ligase Mdm2, which targets the tumor suppressor pro-



tein p53 for degradation, show that Mdm2 inhibition can be attained by blocking interaction with its substrate. Yang *et al.* show that a stable complex forms between TRAF6 and Akt, suggesting that this approach may be a good way to block TRAF6-mediated Akt activation. The potential effectiveness of this approach for tumor therapy is highlighted by the point in the signaling cascade at which TRAF6 contributes to Akt activation—downstream of common mutations observed in the clinic that affect phosphatidylinositol 3-kinase (PI3K) or the phosphatase PTEN, both of which cause hyperactivation of Akt. In support of this, the tumor cell line depleted of TRAF6 that was injected into mice by Yang *et al.* did not express PTEN and displayed strong Akt activation.

TRAF6 could be used to augment the effectiveness of rapamycin analogs (rapalogs), drugs that inhibit the mammalian target of rapamycin complex 1 (mTORC1). Rapalogs are approved for limited antitumor therapy because they may temporarily stabilize tumors in clinical trials but rarely elicit a full response in terms of tumor ablation. Preclinical studies indicate that rapalogs have a cytostatic effect on tumors, due at least in part to increased Akt activation, because a negative feedback loop that normally prevents PI3K signaling is lost. As Yang *et al.* show, cells lacking TRAF6 display increased spontaneous apoptosis (programmed cell death). Thus, TRAF6 inhibition in conjunction with rapalogs could shift the response of tumors to rap-

alogs from cytostatic to cytotoxic, increasing the efficacy of these drugs in cancer therapy.

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PHYSICS

Coupling Strongly, Discretely

James Hone¹ and Vikram V. Deshpande²

The fields of electronics and mechanics have made impressive progress toward true quantum mechanical devices. Through improvements in device performance and measurement techniques, nanoelectromechanical systems (NEMS) have enabled high-sensitivity detection of charge, mass, and spin, and have steadily approached the quantum limit of mechanical motion (1). Similarly, the ability to manipulate individual electrons in quantum dots has led to developments in solid-state quantum computing (2). On pages 1107 and 1103 of this issue, Lassagne *et al.* (3) and Steele *et al.* (4) bring together these two fields to study the influ-

ence of charge transport on nanomechanical motion in high-performance carbon nanotube mechanical resonators that simultaneously act as quantum dots. They find that the resonant frequency and dissipation in the nanotubes are both highly sensitive to the charge state at the level of single electrons.

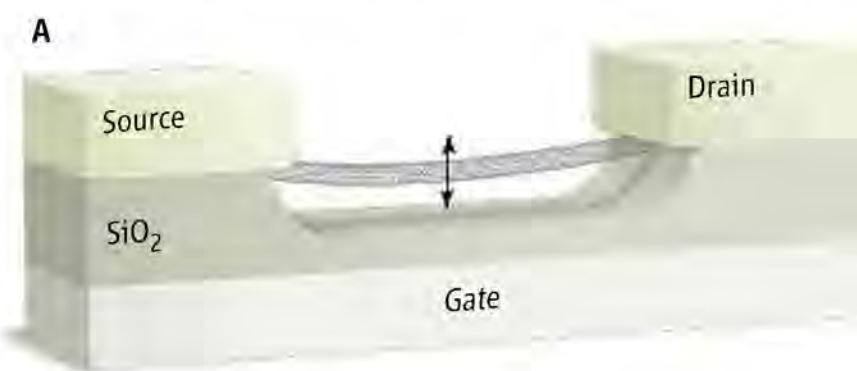
Carbon nanotubes are a model system for nanoelectronics. Adding even a single electron to this small system carries a large energetic cost; thus, at low enough temperatures, electrical transport in carbon nanotubes can take place through the tunneling of electrons one at a time. This manifests itself in peaks in the current as a function of the voltage on a nearby gate (which modulates the chemical potential of the nanotube), a phenomenon known as Coulomb blockade. Advances in the growth and fabrication of nanotubes (5) have enabled the development of clean, freely sus-

pended devices that have been used to observe electronic phenomena such as correlated electron states (6, 7) and spin-orbit coupling (8).

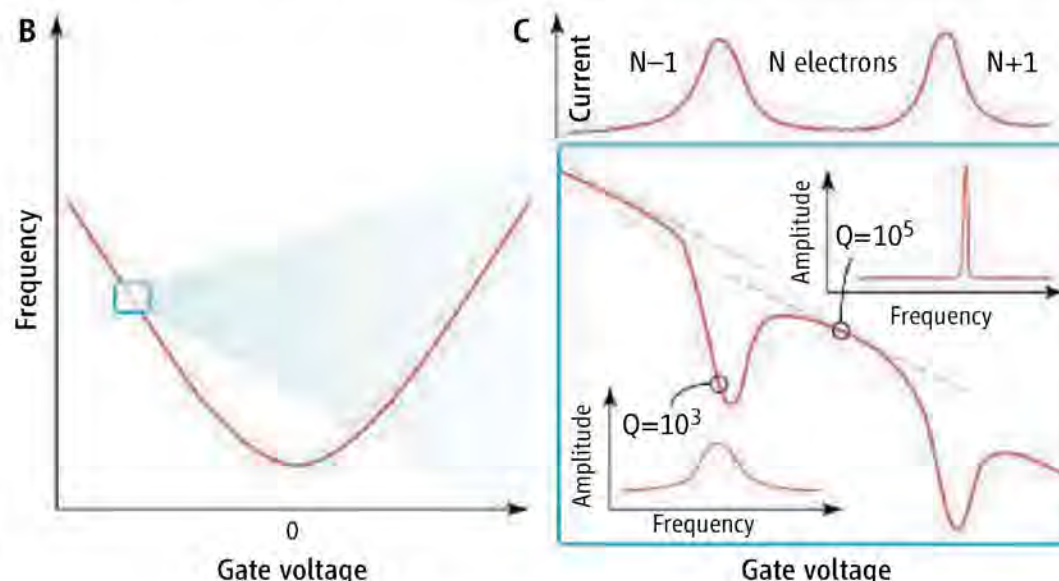
Because of their small size, high stiffness, and low density, nanotubes are also excellent materials for NEMS. But they may also have an additional advantage. They circumvent the surface dissipation mechanism known, in bulk-etched NEMS, to decrease the quality factor Q (which quantifies the sharpness of the resonance peak) with decreasing device size. However, nanotubes have until recently shown Q values of only 100 to 1000 (9–12), in keeping with the trend for etched devices. Steele *et al.* now show that clean nanotubes do in fact beat the trend, achieving a Q of 100,000 at ultralow temperatures.

The two reports take advantage of these parallel improvements in device performance to examine in detail the coupling

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Electronic vibrations. (A) Schematic of device geometry for single-electron tuning. (B) Tuning resonant frequency with gate voltage. (C) (Top) Single-electron Coulomb blockade oscillations. (Bottom) Tuning resonant frequency with gate voltage at the level of single electrons.



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between the electrical and mechanical properties of nanotube NEMS in the Coulomb blockade regime. In both studies, a nanotube is suspended above a substrate, which acts as a gate (see the figure, panel A). Lassagne *et al.* use an electromechanical mixing scheme that takes advantage of the change in current with gate voltage. This scheme is particularly well suited to the Coulomb blockade regime, in which conductance oscillations lead to a large signal, providing single-atom mass sensitivity (12). Steele *et al.* measure the dc conductance, which is sensitive to the second derivative of the current with gate voltage. Motion of the nanotube modulates the capacitance to the gate, and therefore the charge state (and conductance) of the nanotube.

As voltage is applied to the gate, electrostatic force induces tension in the nanotube and increases the resonant frequency (see the figure, panel B). However, the frequency does not change smoothly, but shows discrete jumps (see the figure, panel C), which are correlated with the charge state as deter-

mined by the Coulomb blockade measurement. This occurs because the charge on the nanotube, and therefore the electrostatic tension, changes in discrete amounts; Steele *et al.* term this effect "single-electron tuning," as the mechanical analog to single-electron tunneling. In addition, the resonance softens and broadens at each jump. Both effects are a direct result of the fluctuating charge at the boundary between states with N and $N \pm 1$ electrons.

Although the discussion so far has addressed the effects of charge transport on the mechanical measurement, the opposite also can be interesting. Steele *et al.* find that in the regime of strong coupling to the leads (rate of tunneling larger than resonant frequency), electron tunneling may spontaneously drive the nanotube into resonance, and consequently distort the dc transport features.

The work of Lassagne *et al.* and Steele *et al.* beautifully demonstrates the rich physics that arises from the coupling of NEMS and electron transport in quantum dots. In addition to the results described in these two stud-

ies, such strong electronic-vibrational coupling may be used to investigate interesting physics such as negative charging energy (13). Moreover, the newly achieved high Q 's make nanotubes very attractive candidates for detecting the quantum limit of motion and subsequent manipulation of quantum states at macroscopic scales.

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ATMOSPHERE

Antarctica's Orbital Beat

Peter Huybers

Alternating glacial and interglacial conditions have dominated Earth's climate for at least the past 800,000 years (1, 2). Such a global rhythm of glaciation is surprising—at least if summer solar radiation controls glaciation (3)—because variations in Earth's orbit cause opposite changes in the intensity of northern and southern summer radiation. Deciphering the origins of the orbital period variations found in Antarctic proxies of climate may tell us why glaciations are global.

Earth's orbit around the Sun is not steady. The tilt of its spin axis varies with a period of 41,000 years, the eccentricity of its orbit changes at time scales of 100,000 to 400,000 years, and the orientation of the eccentric orbit precesses with respect to the seasons about once every 21,000 years. One implication of the orbital geometry is that at the time when precession aligns Earth's closest approach to the Sun (perihelion) with Northern Hemisphere summer, Earth is farthest away from the Sun (at aphelion) during Southern Hemisphere summer. But if the north and south are alternately

near and far from the Sun during summer, why has glaciation been globally synchronous?

A clue lies in Antarctica's ice, as illustrated by the δD record from Dome C (see the figure) (4). δD is the normalized deuterium to hydrogen ratio of the ice and is sensitive to air temperature over Antarctica, as can be roughly understood in that colder temperatures lead to greater distillation of heavy isotopes out of atmospheric moisture. The exact δD of the snow accumulated at Dome C depends on detailed evaporation and precipitation histories (5), but similarities between the δD variability and other Southern Hemisphere climate records (6, 7) suggests that this signal represents regional and hemispheric climate variations.

The Dome C δD record (4) indicates that Antarctic temperature increases with the tilt of Earth's spin axis. This is as expected, because greater tilt increases the annual incoming solar radiation (insolation) at high latitudes. More puzzling is that temperature also seems to be higher when aphelion occurs during Antarctic summer (1, 2, 4, 6). This contrasts with the Northern Hemisphere, which warms when perihelion aligns with northern summer (2, 3). The northern response can be understood as more intense summer insolation reducing ice cover,

What do Antarctic ice core records really record?

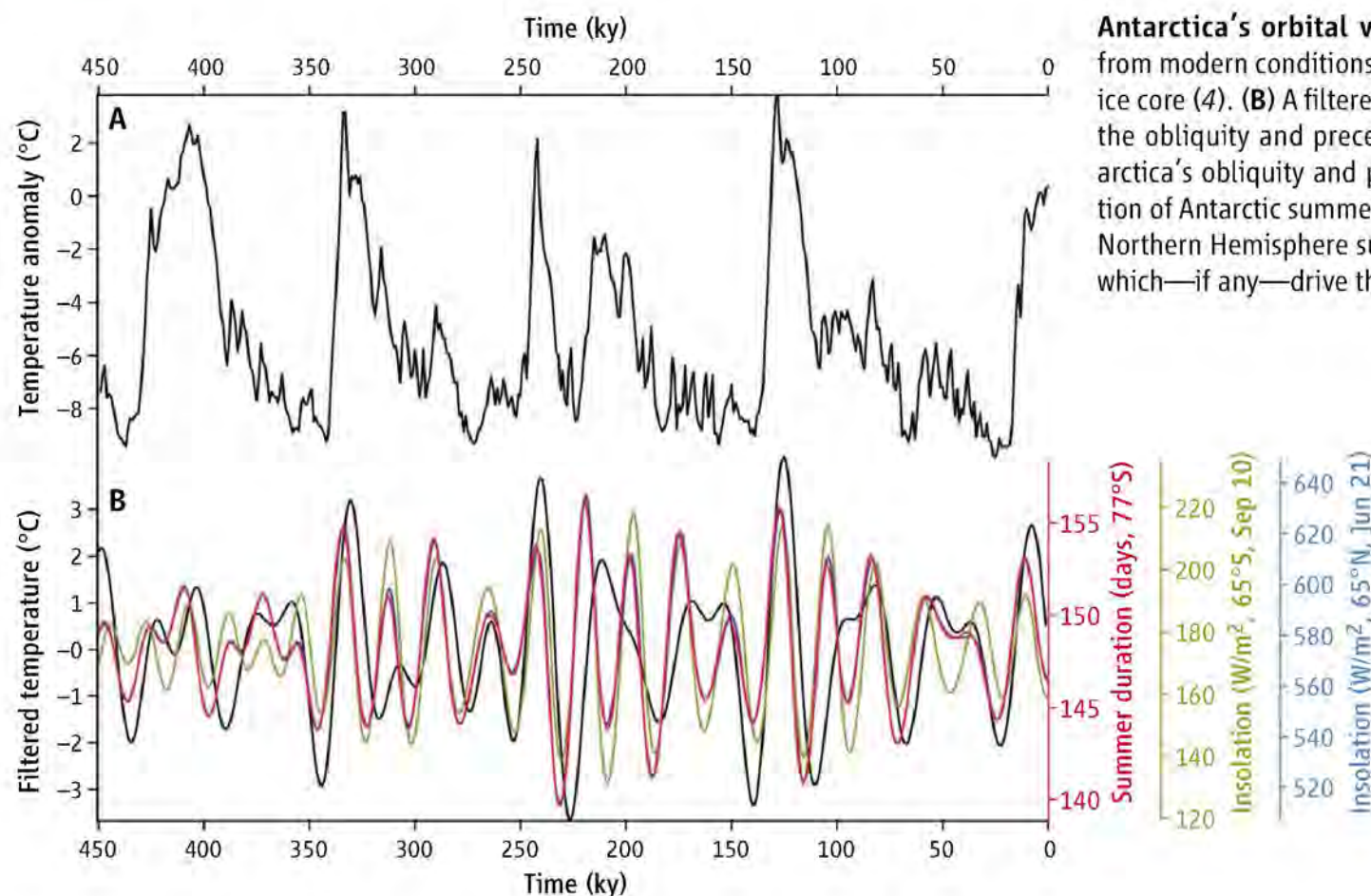
leading to lower surface reflectivity and higher temperatures (2, 3), but what mechanism governs the southern temperature response?

The answer to this question may also tell us why glacial/interglacial cycles are global. There are at least five possibilities.

Perhaps the most basic prediction can be traced from Milankovitch (3), who used simple radiative equilibrium calculations to explore how orbital variations influence temperature and ice volume. He dismissed Antarctica as too cold for changes in southern insolation to influence its ice volume, but applying his radiative equilibrium approach to mean annual temperature does suggest that Antarctica will be warmest when aphelion coincides with Southern Hemisphere summer (8). Aphelion is associated with less intense summer insolation, but it also corresponds to a longer summer and shorter winter, as follows from Kepler's second law. Simple radiative equilibrium indicates that the longer summer more than compensates for a lower intensity, giving temperature variations that are consistent with—albeit smaller in amplitude than—those derived from the ice core records (9).

Alternatively, if increased Southern Hemisphere spring insolation drives a reduction in sea

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Antarctica's orbital variations. (A) Temperature anomalies away from modern conditions derived from δD isotopic ratios in the Dome C ice core (4). (B) A filtered version of the temperature record highlights the obliquity and precession period variability in temperature. Antarctica's obliquity and precession period variability mimics the duration of Antarctic summer, Southern Hemisphere spring insolation, and Northern Hemisphere summer insolation (17), raising the question of which—if any—drive the observed changes?

ice, then heat transport into the interior of Antarctica is expected to increase and cause temperature changes like those inferred from the ice core records (10). The intensity of Southern Hemisphere spring insolation is closely related to the duration of summer, and the two quantities are similar when suitably defined (11); as a result, signal processing alone will not discern between these scenarios.

A third possibility is that Antarctic climate marches to the beat of northern insolation (2, 6). The intensity of northern summer insolation might influence Antarctica by changing cross-equatorial atmospheric or oceanic heat transports, although such a causal mechanism must be reconciled with evidence that southern climate changes occur slightly earlier than those in the north (2).

Atmospheric carbon dioxide (CO_2) concentrations also vary with obliquity and precession, but as for Antarctic temperature, the exact orbital forcing that drives these changes remains unclear. Regardless, CO_2 is observed to amplify Antarctic orbital period temperature variability, accounting for perhaps as much as half the amplitude (12). Furthermore, CO_2 is well mixed in the atmosphere and appears to be a good candidate for orchestrating global climate changes.

The relative importance of each of these mechanisms is still debated, although their basic outlines have been known from Southern Hemisphere marine proxies for at least 25 years (1). More recently, attention has turned to a fifth possibility: that ice core proxies unevenly record the seasonal cycle of temperature.

If temperature is unevenly recorded across the seasons, the orbital influence on seasonality

will tend to appear in the record as precession- or obliquity-period climate variability (13). For example, Laepple recently showed (14) that as a result of lower summer accumulation rates on the East Antarctic Plateau, fall, winter, and spring temperatures are more heavily recorded in ice cores than are summer temperatures. In this case, even if orbital variations do not actually influence Antarctica's annual average temperature, the uneven recording of the seasonal cycle is expected to result in orbital period variations in δD similar to those observed. Furthermore, Hutterli recently suggested (15) that sublimation substantially influences the isotopic composition of Antarctica ice. Perhaps Antarctica's orbital beat tells us more about how the seasonal cycle gets recorded than about long-term changes in temperature, although similarity between ice core records and Southern Hemisphere marine proxy records (2, 7) suggests that the orbital signal is not wholly an artifact of the recording process.

Arguments then exist that the orbital variability recorded in Antarctic ice core proxies relate to the seasonal distribution of Southern Hemisphere insolation or to Northern Hemisphere summer insolation; and that the signal could indicate temperature, the seasonal cycle of accumulation, or post-depositional effects. This confusion stems from the fact that many aspects of the insolation forcing have essentially identical variability. The fact that the orbital bands account for only a few of the $\sim 10^\circ C$ changes between glacial and interglacial conditions (16) does not help either. Coming up with orbital scenarios that look like the Antarctic record is too easy. If we are to use Antarctica's orbital beat to better understand

the orchestration of global changes in glaciation, we must first decipher which elements of the climate system are in play and how their responses get recorded in Antarctica's ice.

Some promising lines of research include analyzing the modern seasonal cycle of snow accumulation and its isotopic composition (14), along with postdepositional effects (15), to better constrain the environmental controls on ice core proxies. Inclusion of water isotopes and other proxies in numerical simulations of climate (5)

will push our understanding of the climate record forward. Further integration of the Antarctic record with other continental and marine proxies (7) will also prove useful in synthesizing Antarctica's orbital beat into the full climate song.

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PUBLIC HEALTH

Report: Shrinking Health Workforce Risks U.S. Outbreak Response

Although the country's top health officials say that they have made extensive preparations for this year's H1N1 flu season, a new report from AAAS warns that a looming shortage of infectious disease experts could hamper the U.S. response to future pandemics.

In the next decade, nearly half of the public health department workforce will be eligible to retire, and physician and nurse shortages will strain the resources necessary to prepare and respond to an outbreak. The next generation of public health workers is not being trained at a commensurate rate to fill these vacancies, and the country lacks a strategic plan to educate these responders, the report says.

"As we develop new programs for infectious disease prevention and response, we also need to make sure that there is a sizable and well-prepared workforce to implement those initiatives," said report coauthor Kavita Berger, project director at the AAAS Center for Science, Technology and Security Policy.

The report—"Workforce Development: Preparing the Next Generation against Infectious Disease Threats"—is the outcome of a 26 May workshop at AAAS that brought together public health educators and experts in biosecurity and public health law. The workshop, organized by the center and the AAAS Scientific Freedom, Responsibility and Law Program, was followed by a 2 July briefing at AAAS at which top public health officials focused on responding to the H1N1 virus.

At the briefing, speakers were cautiously optimistic about the government's vaccine development and flu response programs. Dr. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health, said that the federal government has been aggressively preparing for an infectious disease outbreak for the past several years.

Rear Admiral Anne Schuchat, an assistant U.S. surgeon general, said that one of the largest challenges the government faces is communicating the risks and steps to control the outbreak, while correcting misinformation.

Meeting such challenges could become increasingly difficult as the infectious disease workforce dwindles. But according to the new report, even today's health workers may not be



Future threat. Nearly half of the U.S. public health department workforce could retire in the next decade.

properly trained in fields such as risk communication and disaster planning.

A decade of threats, from the 2001 anthrax attacks to the emergence of SARS and avian flu, led to a proliferation of education programs for outbreak responders. But the plans have been developed without a common guiding curriculum, the report concluded, and few have been rigorously evaluated to determine their effectiveness.

"It is surprising to me, eight years after the anthrax attacks ... that there are still so many questions about the most effective way of training people," said workshop participant Julie Fischer, a senior associate at the Henry L. Stimson Center's Global Health Security program. "We're still struggling to find a way to take the best practices and share them with the right stakeholders."

Training differences were illuminated during the anthrax attacks, when the first case was detected in Florida by a doctor who had just returned from a bioterrorism responder program at the U.S. Centers for Disease Control and Prevention. The doctor accurately diagnosed the disease and contacted a local hospital, state public health laboratories, and the CDC for confirmation. New York City health officials were much slower in confirming later anthrax attacks, the report noted, in part because the officials did not receive the same training.

The front lines of an outbreak include responders from a variety of backgrounds, and the report encourages cross-training of professionals to better anticipate outbreaks and respond to a disease's far-reaching and sometimes unexpected effects.

"Communities worry about leaks into the local environment. Local responders must be prepared to treat persons exposed to toxic pathogens. Lawyers and local government officials need to balance individual rights such as the freedom to travel with the possible spread of a lethal infection," said Mark S. Frankel, director of the Scientific Freedom, Responsibility and Law Program.

As the H1N1 pandemic demonstrated again last fall, infectious disease preparedness is a worldwide concern. In 2007, the United States joined 193 countries in adopting the World Health Organization's International Health Regulations 2005. Under the agreement, the U.S. is legally obligated to improve its public health capacity as part of an international effort to fight infectious disease.

The report recommends international standards and increased funding for training infectious disease workers in developing countries. "These are the places that have the greatest risk of emerging diseases ... and they are the places that generally have the smallest skilled lab workforce," said Fischer. Robust global cooperation, she noted, could lessen the impact of future pandemics.

Read the full report at www.aaas.org/go/workforce/.

—Becky Ham and Benjamin Somers

SCIENCE POLICY

AAAS Issues Guide to S&T Policy Studies

AAAS has updated and expanded its Guide to Graduate Education in Science, Engineering and Public Policy, a valuable resource for the growing corps of students and professionals who are considering a career in public policy as a way to address climate change, energy, and other global issues.

The 4th edition of the guide—available for free online—includes a list of over 40 schools in the United States and abroad that offer special graduate programs in science, engineering, and public policy (SEPP), along with a discussion of possible career paths, answers to frequently asked questions, and a wide-ranging collection of helpful links.

"The institutions and programs listed in the SEPP Guide are essential to meeting the growing demand for individuals who can bridge the two worlds of science and policy," said Al Teich, director of Science and Policy Programs at AAAS.

Find the guide at www.aaas.org/spp/sepp.

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Antibiotics for Emerging Pathogens

Michael A. Fischbach¹ and Christopher T. Walsh²

Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals and the community. New antibiotics are needed to combat these bacterial pathogens, but progress in developing them has been slow. Historically, most antibiotics have come from a small set of molecular scaffolds whose functional lifetimes have been extended by generations of synthetic tailoring. The emergence of multidrug resistance among the latest generation of pathogens suggests that the discovery of new scaffolds should be a priority. Promising approaches to scaffold discovery are emerging; they include mining underexplored microbial niches for natural products, designing screens that avoid rediscovering old scaffolds, and repurposing libraries of synthetic molecules for use as antibiotics.

There is a perpetual need for new antibiotics: Whereas most drugs will be just as effective in the future as they are today, the inevitable rise of resistance will erode the utility of today's antibiotics (1). Two factors exacerbate this supply problem by creating unique disincentives for antibiotic development (2). First, antibiotics are used in smaller quantities than other drugs. Prescriptions for chronic illnesses can last years or decades, whereas a standard course of antibiotics lasts only weeks; therefore, antibiotics yield lower revenues than most drugs. Second, whereas most newly approved drugs can be prescribed to all who would benefit, the use of a newly approved antibiotic may be restricted to the treatment of serious bacterial infections. The result is a quandary: Resistance is on the rise while antibiotic discovery and development are on the decline (3, 4).

The unfavorable economics of antibiotic development have had a chilling effect on industrial discovery programs, and policy-based efforts to reverse this decline deserve attention (3). This perspective focuses on a different, yet no less formidable, challenge: finding new classes of antibiotics.

On the face of it, antibiotic discovery would seem to be straightforward. The goal is to kill an organism that is only distantly related to humans; unique, essential targets should be abundant, and novel antibiotics with low toxicity should be easy to find. Yet, the history of antibiotic development suggests otherwise. Since the early 1960s, only four new classes of antibiotics have been introduced, and none of these has made a major impact yet; the ~\$30 billion global antibiotics market is still dominated by antibiotic classes discovered half a century ago. Since then, most "new" antibiotics have been chemically tailored derivatives of these well-worn scaffolds. In this review, we

argue that the rise of resistant pathogens should redouble our focus on discovering not just new antibiotics, but new classes of antibiotics. We then highlight some promising approaches to scaffold discovery: mining underexplored microbial niches for natural products, designing screens that avoid rediscovering old scaffolds, and repurposing libraries of synthetic molecules for use as antibiotics.

A New Generation of Resistant Pathogens

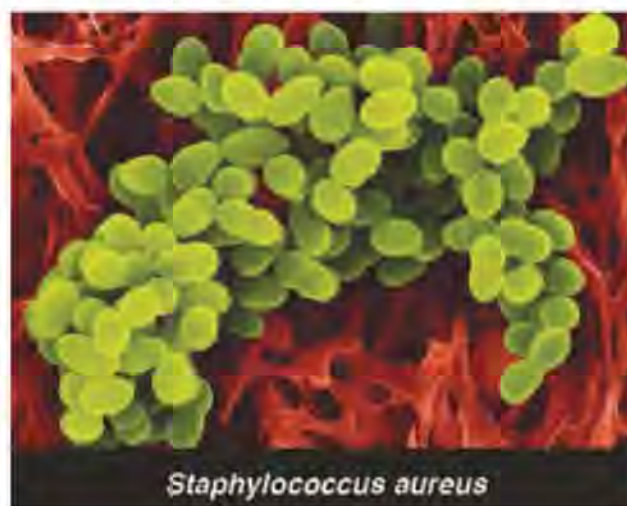
Three classes of antibiotic-resistant pathogens are emerging as major threats to public health (Fig. 1). First, methicillin-resistant *Staphylococcus aureus* (MRSA) is estimated to cause ~19,000 deaths per year in the United States (5). Apart from their high mortality rate, MRSA infections lead to an

estimated \$3 billion to \$4 billion of additional health care costs per year. Furthermore, the rising prevalence of MRSA increases the likelihood that vancomycin-resistant *S. aureus* (VRSA) (6)—just as deadly as MRSA but more challenging to treat—will become a new scourge in hospitals.

Pathogens from the second class, multidrug-resistant (MDR) and pandrug-resistant (PDR) Gram-negative bacteria, are less prevalent than MRSA, but they pose the grave threat of infections that are truly untreatable (7). These strains of *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are resistant to some (MDR) or all (PDR) of the antibiotic classes commonly used to treat Gram-negative bacteria: penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides, tetracyclines, and polymyxins (7). Prospects for finding new antibiotics for Gram-negative pathogens are especially poor: Their outer membrane blocks the entry of some antibiotics, and efflux pumps expel many of the remainder.

The third class comprises MDR and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB), which are a rising threat in the developing world (8). MDR-TB treatment requires a 2-year course of antibiotics with serious side effects; XDR-TB is even more difficult to cure and often fatal (9). Cases of MDR-TB and XDR-TB have been reported in the United States and other developed countries.

In spite of the rise of resistant pathogens, the rate of new antibiotic approvals is dropping. Where will new antibiotics come from? In the past, this



Staphylococcus aureus



Mycobacterium tuberculosis



Acinetobacter baumannii



Pseudomonas aeruginosa

Fig. 1. Multidrug-resistant strains of these bacterial pathogens are on the rise. [Credit: Dennis Kunkel Microscopy, Incorporated]

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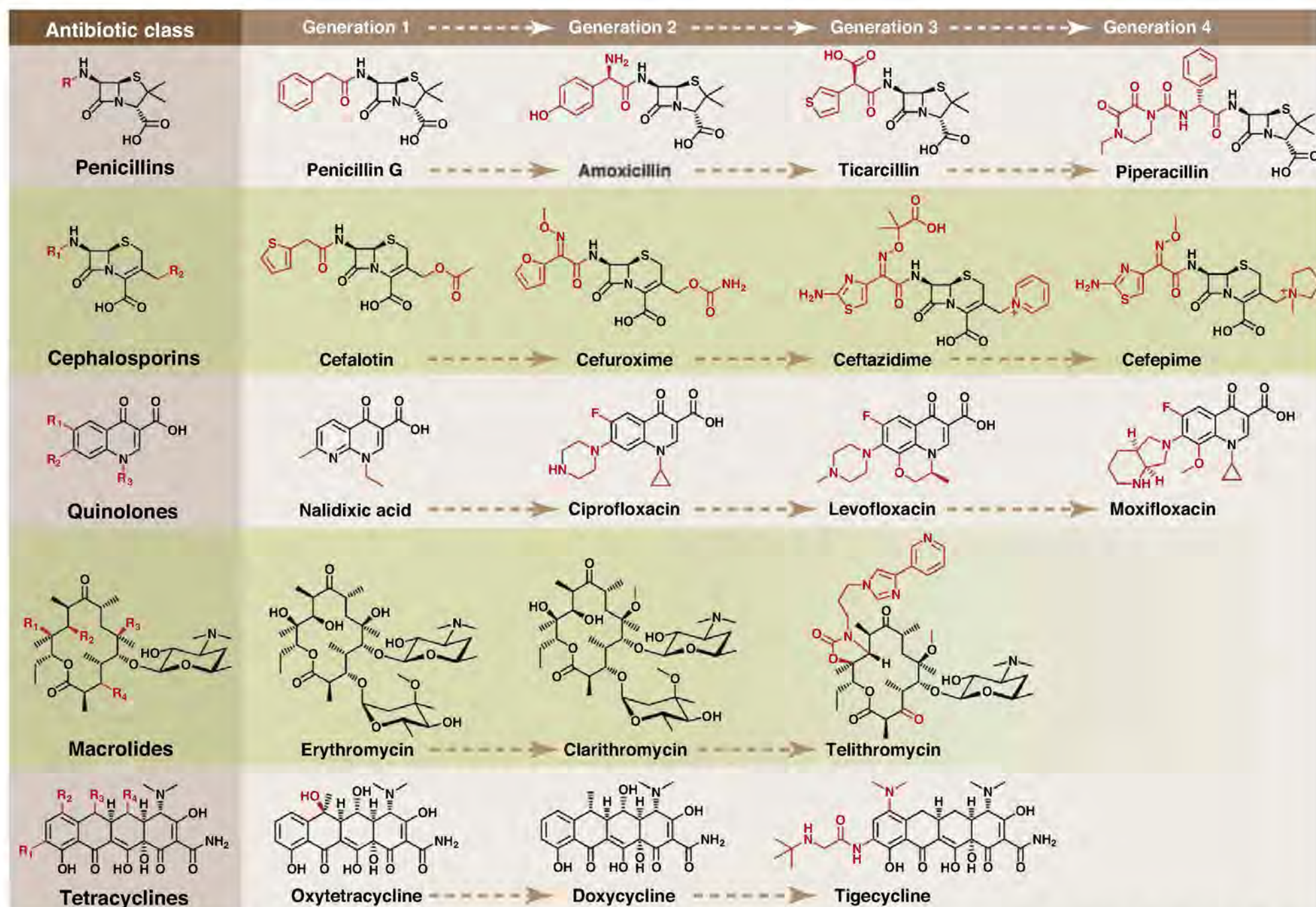


Fig. 2. Synthetic tailoring is widely used to create successive generations of antibiotic classes. Scaffolds are colored black; peripheral chemical modifications are colored red. The quinolone scaffold is synthetic, whereas the other scaffolds are natural products.

question has mostly been answered through synthetic tailoring of a small group of “scaffolds.”

Few Scaffolds, Many Generations of Tailoring

Members of each antibiotic class share a common core structure, or scaffold. For example, the cephalosporins share a β -lactam embedded in a fused 4,6-ring system (Fig. 2). Most chemical scaffolds from which today's antibiotics are derived were introduced between the mid-1930s and the early 1960s (Fig. 3). Aside from the introduction of carbapenems in 1985, all antibiotics approved for clinical use between the early 1960s and 2000 were synthetic derivatives of existing scaffolds. Just four such scaffolds—cephalosporins, penicillins, quinolones, and macrolides—account for 73% of the antibacterial new chemical entities filed between 1981 and 2005 (10).

During synthetic tailoring (Fig. 2), the core of the antibiotic is left intact, preserving its activity, but the chemical groups at its periphery are modified to improve the drug's properties. New generations are often designed to be active against pathogens that have become resistant to the previous generation. For example, second- (11) and third-generation (12) cephalosporins like cefaclor and ceftazidime are more resistant to destruction

by the resistance enzyme β -lactamase, and they can penetrate the Gram-negative outer membrane more effectively. When new β -lactamases emerged that can cleave third-generation cephalosporins, pharmaceutical companies developed fourth-generation molecules, like cefepime, that are less susceptible to cleavage by these enzymes (13). Cephalosporins and other semisynthetic antibiotics account for 64% of the new chemical entities filed between 1981 and 2005 (10), suggesting that incremental synthetic tailoring of natural scaffolds has become the predominant mode of antibiotic discovery. The most useful scaffolds have therefore been those that are easy for medicinal chemists to tailor; this allows many derivatives to be synthesized and tested for improved properties.

Organic synthesis plays two other key roles in antibiotic discovery. First, scaffolds like the quinolones and oxazolidinones are derived entirely from chemical synthesis; these fully synthetic scaffolds account for an additional 25% of the antibiotic new chemical entities. Second, some natural scaffolds like carbapenems can now be produced entirely by organic synthesis, expanding the scope of accessible scaffold modifications.

The interplay between semisynthesis and total synthesis—and the ability of synthetic modifi-

cations to unlock the therapeutic potential of a scaffold—are exemplified by the tetracyclines. Resistance to this class of 30S-targeting antibiotics is mediated in part by a widely distributed gene encoding an efflux pump. Semisynthetic modifications to the tetracycline scaffold yielded the glycylcycline tigecycline (Fig. 4) (14). This third-generation molecule (Fig. 2) is no longer a substrate for the efflux pump, restoring its activity against tetracycline-resistant pathogens. A fully synthetic route to the tetracyclines (15) makes it possible to modify scaffold positions that are difficult to modify semisynthetically, further broadening the range of accessible derivatives.

Making incremental improvements to existing scaffolds is a good short-term strategy for refilling the antibiotic pipeline, but a presumably more sustainable way to combat resistance is to discover new scaffolds. Their utility will depend on three criteria: spectrum of activity against Gram-positive and Gram-negative pathogens, lack of cross-resistance to existing drugs, and amenability to generations of synthetic tailoring.

Next-Generation Scaffolds: Natural Products

More than two-thirds of clinically used antibiotics are natural products or their semisynthetic derivatives

(10). It is therefore troubling that natural product discovery efforts have waned in recent years (16); this decline is due in part to a rising rate of scaffold rediscovery (17) and the accompanying difficulty in finding new antibiotics. Recent efforts to search new modalities—underexplored ecological niches, unmined bacterial taxa, and the genomes of even well-studied bacteria—have yielded novel molecules, whereas new screening strategies have begun to circumvent the time-consuming problem of rediscovery (18).

New places to look. Most natural product antibiotics have come from soil actinomycetes, reflecting the historical bias of pharmaceutical screening programs toward these easily collected and cultured bacteria (1). Searches of underexplored ecological niches and bacterial taxa have revealed new molecules. Marine niches are particularly promising; for example, a deep-sea sediment sample yielded an actinomycete that produces the abyssomicins (19), a new antifolate scaffold (Fig. 5). Terrestrial and marine symbioses are also promising ecological niches; recent efforts to study bacterial symbionts of insects, ascidians, and fungi have yielded many new natural products (20–23). Among underexplored bacterial taxa, myxobacteria are particularly prolific natural product producers, and their continued mining holds much promise for the discovery of new antibiotic scaffolds (24).

The genome sequences of a handful of actinomycetes and myxobacteria have revealed that these bacteria generally harbor >25 gene clusters encoding secondary metabolites. Given that only one to four natural products are known from a typical bacterium under various culture conditions, researchers may as yet have discovered only 10% of natural products from screened strains and just 1% of molecules from the global consortium of microbial producers (25). Taking this lesson to heart, several industrial and academic groups have carried out bioinformatics-based efforts to

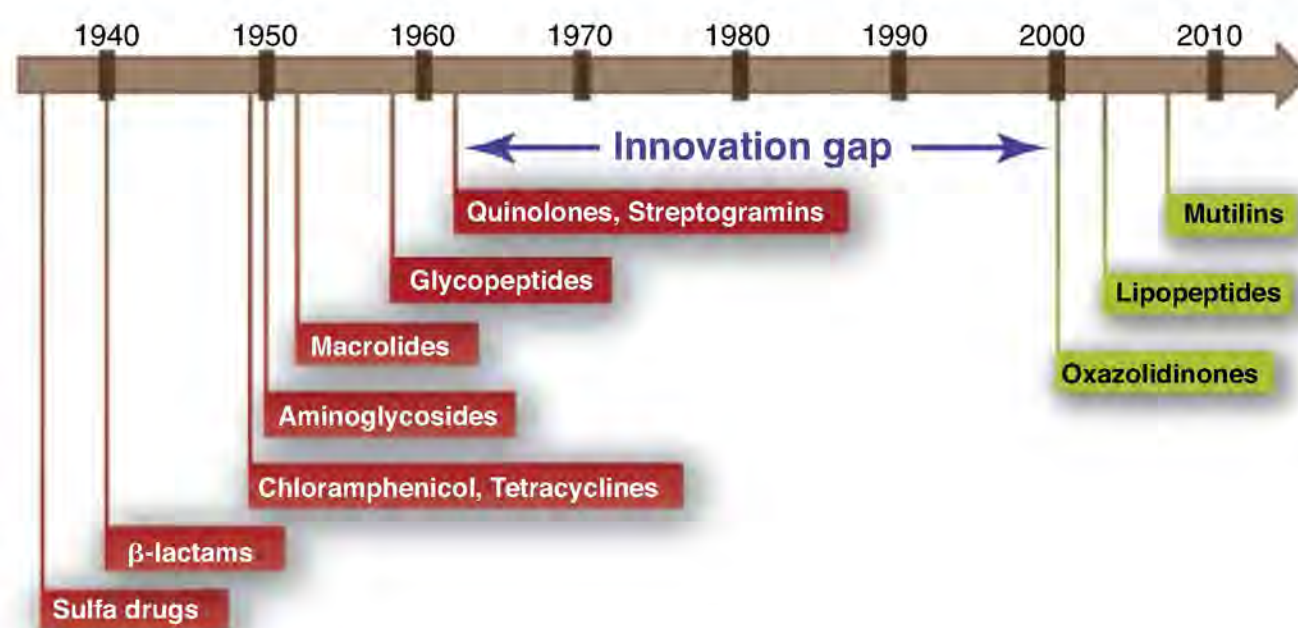


Fig. 3. Between 1962 and 2000, no major classes of antibiotics were introduced.

mine bacterial genomes for new natural products (26, 27). Ecopia Biosciences (now Thallion Pharmaceuticals) has had particular success with their genome-scanning approach, including the discovery of ECO-0501, a new antibiotic scaffold (28) (Fig. 5). If the throughput of these genomics-based approaches to natural product discovery can be scaled up efficiently, their contribution to antibiotic discovery will be increasingly important.

Lastly, some promising candidate scaffolds for development may already be known. The founding members of the three most recently introduced antibiotic classes—mutilins, lipopeptides, and oxazolidinones—were each discovered at least 2 decades before they were introduced. Old patent literature seems a good place to start; on the basis of a 1985 patent from Eli Lilly, a group from Bayer recently isolated a series of acyldepsipeptide antibiotics that activate the bacterial chambered protease ClpP, leading to uncontrolled proteolysis and cell death (29) (Fig. 5). Focusing development

efforts on known but underexplored scaffolds can mitigate the risk of a costly and time-consuming de novo discovery program.

Combating rediscovery. Out of 1000 randomly selected actinomycetes, about 10 will produce streptomycin, and 4 will produce tetracycline (30). If extracts from these strains are screened against an indicator organism, most hits from the screen will be unhelpful rediscoveries. Two new screening strategies are beginning to circumvent the problem of rediscovery (16). First, researchers at Cubist have developed a strain of *E. coli* that harbors resistance genes for the 15 most commonly rediscovered antibiotics (31). Hits from their screening efforts are therefore preselected to be members of novel classes.

Second, a group at Merck has reported a bacterial antisense technology that allows them to knock down the expression of a given *S. aureus* gene, decreasing the amount of the encoded protein to the point that, in principle, it is present in

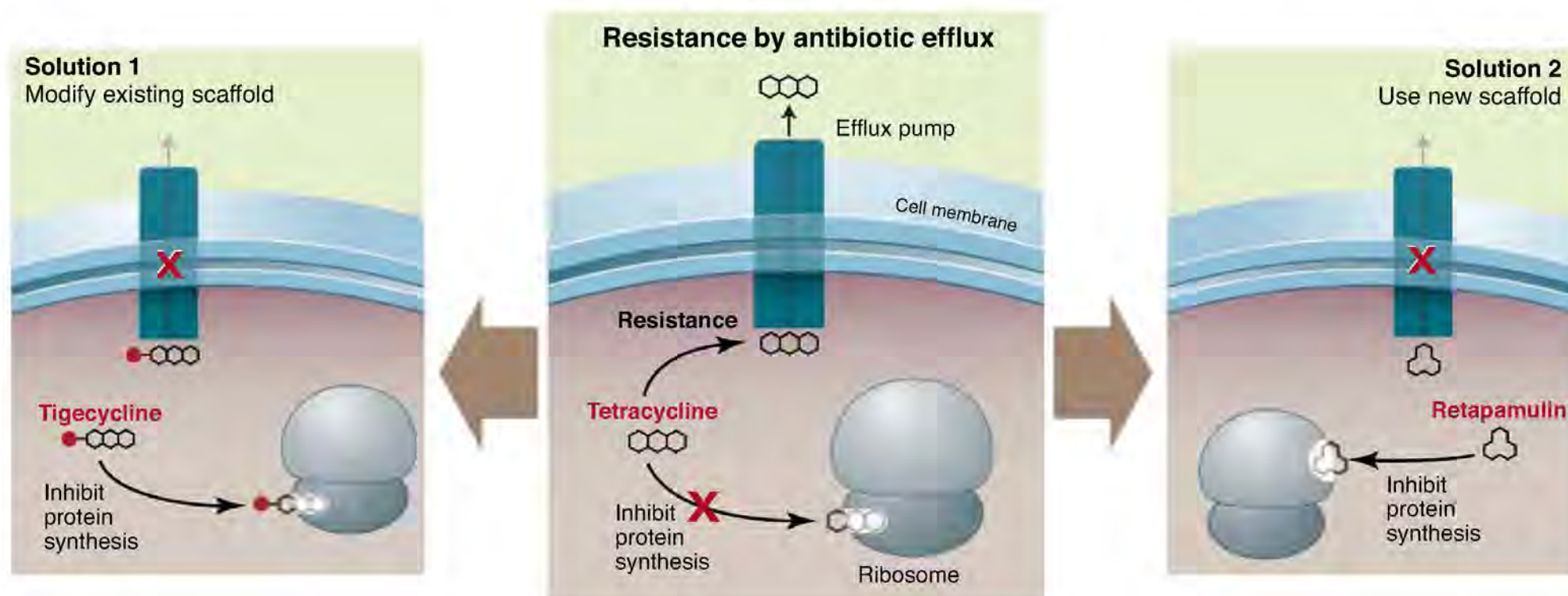


Fig. 4. Surmounting resistance with scaffold alterations. Two ways of overcoming resistance are shown, using tetracycline (center) as an example. First, the tetracycline scaffold can be chemically modified, creating a tetracycline derivative

like tigecycline that is no longer a substrate for the efflux pump (left). Second, a new scaffold like retapamulin, which is not a substrate for efflux and binds to a different site in the ribosome, can be used instead of tetracycline (right).

growth-limiting quantities (32). Using this approach, they discovered platensimycin, the founding member of a new class of fatty acid biosynthesis inhibitors (33) (Fig. 5), as well as several new protein synthesis inhibitory scaffolds.

Next-Generation Scaffolds: Synthetic Molecules

Fully synthetic molecules are a crucial component of the current antibiotic arsenal: The quinolones are highly effective broad-spectrum antibiotics, and the oxazolidinones are of increasing importance in the treatment of Gram-positive pathogens, including MRSA. However, recent efforts—based largely on high-throughput screens of novel targets identified by bacterial genomics—to discover and develop new synthetic scaffolds have not yet been successful (34).

Historically, synthetic scaffolds have originated outside of antibiotic discovery programs. The first drug in the sulfa class of antibiotics, Prontosil, was originally developed as a dye at Bayer, and the first quinolone was nalidixic acid, an intermediate in the synthesis of chloroquine. The oxazolidinones were discovered at DuPont as antibacterials but were originally developed to treat foliage diseases of plants.

Since the late 1990s, the rise of bacterial genomics held the promise of rejuvenating the discovery of synthetic antibiotics (35). The genome sequences of pathogens like *Haemophilus influenzae*, *S. aureus*, *Streptococcus pneumoniae*, and *E. coli* made it possible to identify conserved enzymes that are essential for bacterial growth. These novel targets served as the basis for high-throughput screens of synthetic compound libraries, an approach that has been fruitful in other therapeutic areas. Genomics-based technologies have accelerated the process of identifying targets of existing drugs (36); however, they have not yet yielded new antibiotics (34, 37).

Use external libraries and a whole-cell screen. The success of repurposing synthetic molecules from other development programs (38) and the failure of other approaches hold two important lessons for developing new synthetic antibiotics. First, look outside antibacterial development programs for synthetic libraries to screen. Most pharmaceutical companies have invested considerable resources in synthesizing small molecule libraries for other therapeutic areas. Given the current level of uncertainty about which targets are relevant in an infected host (39) and how antibiotics get into bacterial cells (40), libraries developed for other therapeutic areas may be just as likely to harbor hits as compound libraries developed for antibacterial screening.

Second, unbiased whole-cell screens have fewer pitfalls than other assays. The advantages of target-based screening—knowledge of the target and ease of optimization using a biochemical screen—are outweighed by the disadvantage of having to engineer cell permeability into a scaffold at a subsequent stage of the development process. Technologies like genome-wide expression profiling (36) and whole-genome resequencing of resistant mutants (41) have accelerated the

target identification process; the latter was used to identify adenosine triphosphate (ATP) synthase as the target of the new antimycobacterial agent R207910, the product of a whole-cell screen (41).

Even with a strong hit in a whole-cell antibacterial assay, testing candidates in the right animal model early in development is crucial. In vitro kill assays fail to recapitulate key elements of a

a whole-cell antibacterial assay (38). Miller and co-workers screened a one-million-compound library developed for eukaryotic protein kinase inhibition in an assay of *E. coli* killing, predicting that the low molecular weight ATP-mimetic molecules in the library might inhibit an essential bacterial enzyme and therefore exhibit antibacterial activity. They identified a set of pyridopyrimidines

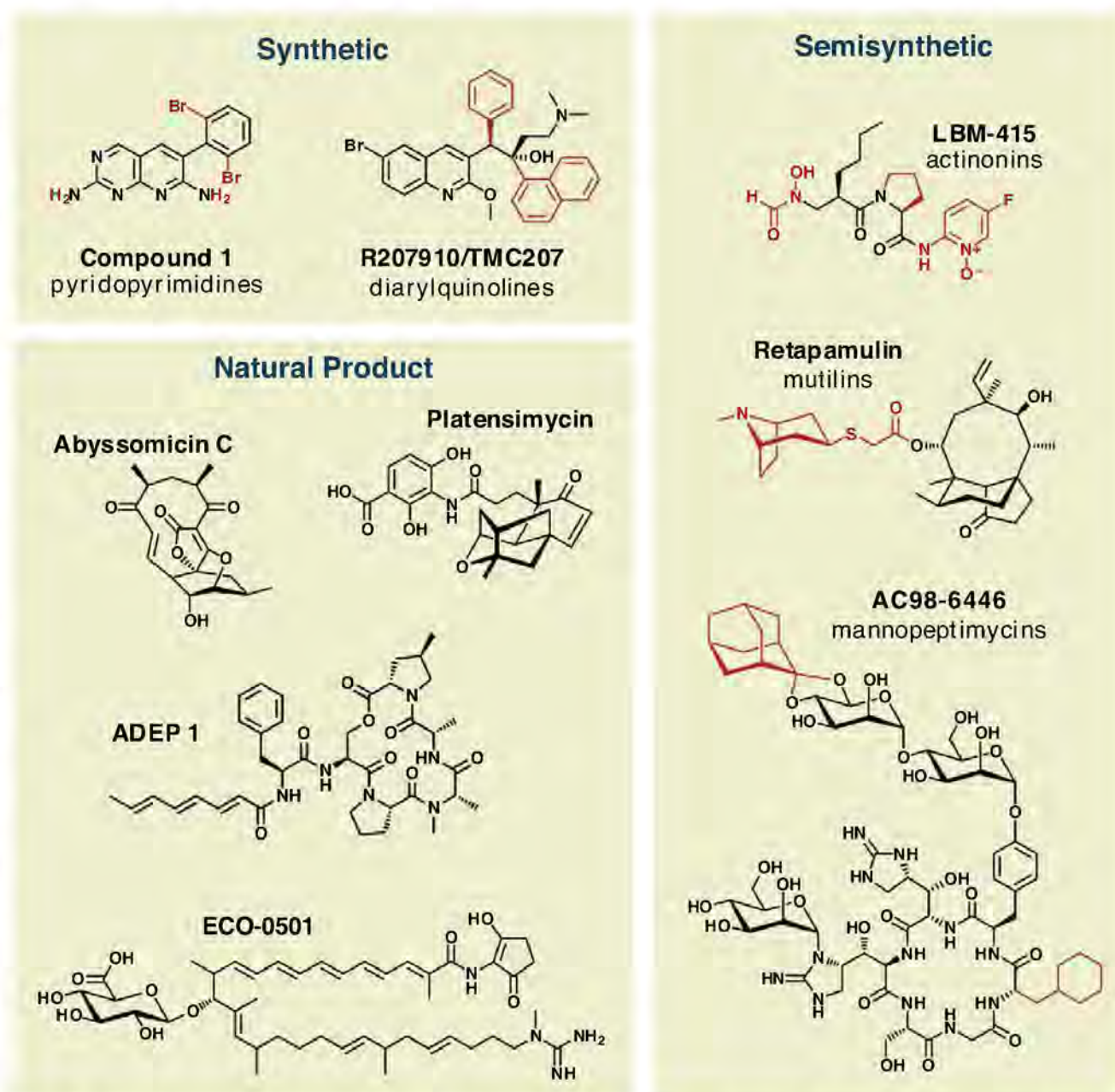


Fig. 5. The chemical structures of new and underexplored antibiotic scaffolds mentioned throughout the text are organized by type into three categories: synthetic, semisynthetic, and natural product. For synthetic and semisynthetic scaffolds, core scaffolds are shown in black and variable positions are shown in red.

bacterial infection, such as the hypoxia and oxidative stress that *M. tuberculosis* experiences in a host (42). A recent report has cast doubt on whether lipid synthesis is a viable target for Gram-positive pathogens; its authors argue that most models of infection fail to account for the fact that lipids in human serum can circumvent the inhibition of fatty acids synthesis (39). Although future experiments will help resolve whether lipid synthesis inhibitors will be useful as drugs for *Staphylococcus* and *Streptococcus*, the mycolic acid pathway is already a well-validated target for *M. tuberculosis*. Any identified fatty acid synthesis inhibitors should therefore be tested against TB rather than being shelved for lack of efficacy against other Gram-positive pathogens.

A recent example of success. A recent report from Pfizer demonstrates the utility of repurposing external compound libraries by screening them in

(Fig. 5) that are subnanomolar inhibitors of the biotin carboxylase subunit of acetyl-coenzyme A (CoA) carboxylase (ACC), acting as competitive inhibitors of ATP binding. These molecules are selective for bacterial ACC over eukaryotic protein kinases and have potent activity against Gram-negative bacteria in vitro and in vivo. Similar efforts using other existing libraries could uncover new targets and scaffolds.

Is There Still a Role for Target-Based Antibiotic Discovery?

The failure of bacterial genomics to validate novel targets or yield new antibiotics has cast doubt on the utility of target-based discovery programs (34, 37). Nevertheless, retooled target-based strategies can play an important role in discovery. Examples include developing novel scaffolds for old targets and grouping new targets by inhibitor class.

A new look at old targets. Most clinically used antibiotics inhibit enzymes from pathways that have been known for decades: peptidoglycan synthesis, ribosomal protein synthesis, folate synthesis, and nucleic acid synthesis and topoisomerization. Future generations of existing scaffolds should continue to have success in the clinic, and these classical targets will thus remain useful. However, a complementary and perhaps more promising strategy is to develop new scaffolds for these targets, thereby avoiding cross-resistance with existing drugs.

For example, the recently introduced mutilin retapamulin (Figs. 4 and 5) targets the 50S subunit of the bacterial ribosome but is unaffected by resistance to other 50S-targeting classes like macrolides (43). Another target that deserves renewed focus is Lipid II; the success of glycopeptide antibiotics like vancomycin bodes well for other Lipid II-binding molecules like the mannopeptimycins (Fig. 5) and lantibiotics (44).

Grouping targets by inhibitor scaffold. To identify new targets, candidates are often grouped by a functional criterion, such as membership in a validated pathway or essentiality for growth in the laboratory. The attendant dangers of single-target bias (34) argue in favor of a strategy that begins with a wider funnel at its early stages.

A different way of grouping targets—by a common inhibitor scaffold rather than by pathway—may not only reveal new targets but also clues about how to inhibit them. For example, ATP-binding enzymes are a group of targets that can be inhibited by ATP-mimetic scaffolds, and they deserve particular attention for two reasons.

First, bacterial genomes encode hundreds of ATP-binding proteins. They include well-validated targets like DNA gyrase, the target of the quinolones, and a host of new or underexplored targets: the chambered protease ClpP (29), ATP synthase (41), aminoacyl-tRNA synthetases, and acyl-CoA carboxylase. The sensor kinase PhoQ is essential for the virulence of *Salmonella* (45), and several widely conserved essential genes encode proteins of unknown function that are predicted to bind ATP (46), suggesting that this class might include a particularly broad range of relevant targets. Insights from outside the antibiotic arena are also important for antibiotics; the observation that Zn-dependent hydrolases are efficiently inhibited by small molecules with Zn-chelating groups has led to the development of inhibitors for a broad range of enzymes, including angiotensin-converting enzyme, histone deacetylases, and matrix metalloproteases. Indeed, semisynthetic derivatives of actinonin—a Zn-chelating natural product that inhibits the Zn-dependent bacterial enzyme peptide deformylase—have been considered as antibiotic candidates (47) (Fig. 5).

Second, Miller and co-workers have demonstrated the feasibility of finding molecules from libraries of ATP-mimetic molecules that are selective for bacterial targets over human targets (48). Screening these libraries in whole-cell assays could simultaneously identify new targets

and new lead compounds with scaffolds that can be optimized synthetically.

A More Inclusionary Approach?

In the heyday of antibiotic discovery, the pool of lead compounds was large enough for pharmaceutical companies to focus on broad-spectrum antibiotics for use as single-agent therapies and shelve compounds that failed these high therapeutic barriers. Today's greater need for new antibiotics may encourage the development of lead molecules with characteristics that, until recently, have been seen as liabilities: narrow activity spectra and high intrinsic resistance rates.

The rule for antibacterial activity spectrum has been "broader is better." However, the challenge of finding new broad-spectrum antibiotics and the rising threat from specific pathogens like MRSA have led to the development and approval of more agents with a narrower spectrum of activity, particularly those that kill Gram-positive but not Gram-negative bacteria. Extending this trend to near its logical limit, two groups recently reported *Staphylococcus*-selective antibiotics: One group used a repurposed series of eukaryotic cholesterol synthesis inhibitors to block the production of the gold pigment staphyloxanthin (49), from which the species name *aureus* is derived; the other group identified inhibitors of the tubulin-like protein FtsZ to block cell division (50). It remains to be seen whether compounds with a spectrum this narrow find a therapeutic niche; one prerequisite for their use would be the availability of rapid diagnostics to identify the etiological agent of infection (51). Such genus-selective agents may have the benefit of sparing more of the endogenous microflora than conventional antibiotics, thereby avoiding complications like secondary *Clostridium difficile* infections.

Most bacterial infections are treated with a single antibiotic, ruling out the use of molecules with high intrinsic resistance rates. However, pairing these compounds into additive or synergistic combinations could rescue candidates formerly thought to be untenable for development. Although development of combination therapies carries the risk of unforeseen toxicity, precedents like amoxicillin-clavulanate and isoniazid-rifampicin-pyrazinamide-ethambutol all argue that antibacterial combination therapies can be quite successful, especially in suppressing the development of resistance. Whether natural or synthetic, broad-spectrum or narrow, single agents or combinations, new scaffolds will be an essential component of a sustainable plan for combating resistance.

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Gene-for-Gene Resistance in *Striga*-Cowpea Associations

Jianxiong Li* and Michael P. Timko†

Witchweeds (*Striga* species), hemiparasitic angiosperms that attack the roots of many important cereals and legumes, constrain crop productivity worldwide. The most variable and widely distributed witchweed is *S. gesnerioides*, which attacks cowpea [*Vigna unguiculata* (L.) Walp.], a major food and forage legume in the Sahel of West and Central Africa (1). Resistance (R) genes specific to several of the seven *Striga* races (2) map to two different linkage groups (3). Resistant cowpea genotypes exhibit two types of defense: a hypersensitive response at the site of parasite attachment, followed by death of the parasite within 3 to 4 days, and growth arrest of the parasite at the tubercle stage of development, characterized by failure of *Striga* to expand its cotyledons. Host genotype and the specific *Striga* race involved in the interaction determine the nature of the resistance response elicited, suggesting that a gene-for-gene resistance mechanism is operating (2, 4).

We identified a simple sequence repeat (SSR-1) (5) (fig. S1A) cosegregating with *S. gesnerioides* race 3 (SG3) resistance. This SSR is present in all cowpea cultivars resistant to SG3; absent in SG3-susceptible genotypes; and not linked with SG1, SG2, or SG5 resistance (fig. S1B). SSR-1 was identified in a cowpea genome sequence read (6) encoding the carboxy-terminal portion of an R protein homolog (fig. S2). We then isolated the full-length gene (named *RSG3-301*; resistance to *S. gesnerioides* race 3 in B301). The sequence predicts an R protein (*RSG3-301*) with a coiled-coil (CC) protein-protein interaction domain at the N terminus, a nucleotide binding site (NBS), and a leucine-rich repeat (LRR) domain at the C terminus. *RSG3-301* is most similar (~54% identity) to Rpg1-b (fig. S2B), a soybean protein conferring resistance to *Pseudomonas syringae* pv. *glycinea* containing the avirulence factor *avrB* (7). Overexpressed yellow fluorescent protein-tagged *RSG3-301* localizes to the peripheral plasma membrane (fig. S3), consistent with its possible role as a guard molecule against *Striga* attachment and penetration.

We used virus-induced gene silencing (VIGS) to reduce transcription of *RSG3-301* in roots of the multirace-resistant cultivar B301 (fig. S4) and then challenged control, mock-treated, and *RSG3-301*-

silenced plants with various races of *S. gesnerioides* (Fig. 1 and fig. S5). In both pot trials and under in vitro conditions, control and mock (empty vector)-treated B301 plants, 24 to 48 hours after parasite attachment, mounted a response to SG3 that was typified by necrosis of the cowpea host root at the point of parasite attachment, concomitant parasite growth arrest, and subsequent parasite death (Fig. 1A and fig. S5). Although the parasite is able to breach the host root cortex, it cannot cross the endodermis and does not make connections with the host vascular system (Fig. 1C). In contrast, *RSG3-301*-silenced B301 plants challenged with SG3 failed to mount a resistance response (Fig. 1B) and allowed *Striga* to penetrate the endodermis and to establish xylem-xylem connections with the host vascular system (Fig. 1D). Neither *S. gesnerioides* races SG2 nor SG5 were capable of parasitizing *RSG3-301*-silenced B301 plants, and SG2 and SG5 seedlings failed to thrive post-

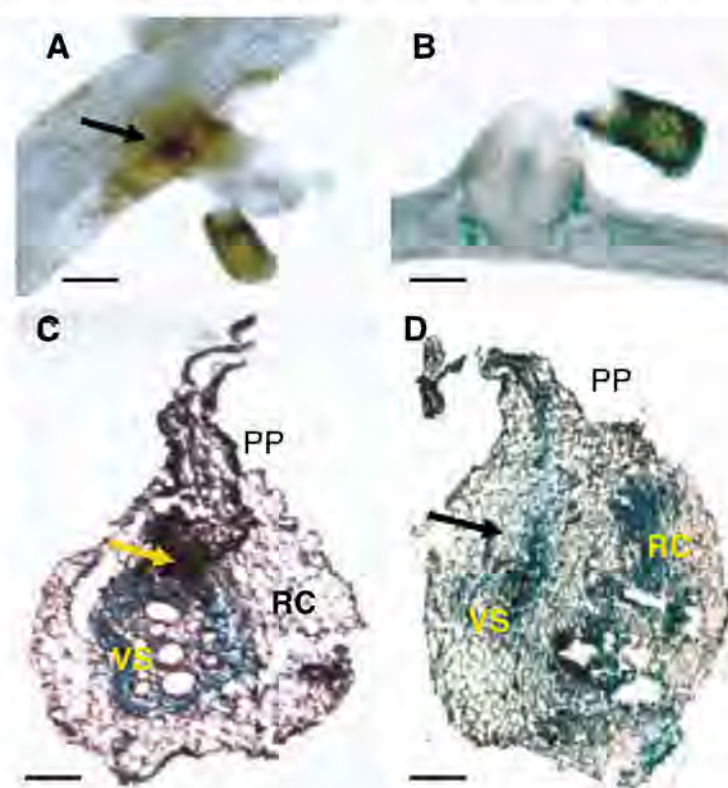


Fig. 1. VIGS-induced suppression of *RSG3-301* enhances *S. gesnerioides* race 3 infection in cowpea roots. Scale bars indicate 100 μ m. (A) SG3-induced necrosis (arrow) at the infection site of mock (empty vector)-treated B301 roots. (B) SG3 tubercle formation on roots of *RSG3-301*-silenced B301 plants. (C) Cross section of mock (empty vector)-treated B301 roots showing failure of SG3 endophyte to breach host root cortex and lack of connection to host vascular system. Arrow indicates interface between the penetration peg (PP) of the invading *Striga* endophyte and the host root cortex (RC). VS, host vascular system. (D) Cross section of *RSG3-301*-silenced B301 plants showing penetrating SG3 endophyte (PP) forming xylem-xylem connection (arrow) to host vascular system (VS).

attachment (fig. S5C and S4D). These data support the conclusion that the *RSG3-301* gene product functions in a race-specific manner.

R proteins function in highly conserved systems for detecting microbial pathogens (8) that involve either direct or indirect recognition of pathogen-derived effectors (9). The identification of *RSG3-301* and its predicted CC-NBS-LRR R protein suggests that plants resist attack by parasitic angiosperms using similar surveillance mechanisms.

Another component of host defenses is RIN4, which serves as a guard protein for the *RSG3-301* homologs *AthRPM1*/*AthRPS2* and *Rpg1-b* found in *Arabidopsis* and soybean, respectively (9). Pathogen-derived effectors modify RIN4, stabilizing its ability to suppress host defenses (8, 9). A RIN4 homolog is present in the cowpea genome (7), but its involvement in mediating a resistance response to *Striga* remains unclear. Although no effectors (avirulence factors) have been identified in *Striga*, evidence of their existence and ability to evolve rapidly comes from the observation that cowpea cultivar B301, initially resistant to all races of *S. gesnerioides* in West Africa including SG4 from Benin, is now susceptible in one location (Zakpota, Benin) where resistance has been overcome (1, 2). Our findings open the door for further detailed exploration of this unique type of plant-plant interaction.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S5
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On the Origin and Spread of an Adaptive Allele in Deer Mice

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Adaptation is a central focus of biology, although it can be difficult to identify both the strength and agent of selection and the underlying molecular mechanisms causing change. We studied cryptically colored deer mice living on the Nebraska Sand Hills and show that their light coloration stems from a novel banding pattern on individual hairs produced by an increase in *Agouti* expression caused by a *cis*-acting mutation (or mutations), which either is or is closely linked to a single amino acid deletion in *Agouti* that appears to be under selection. Furthermore, our data suggest that this derived *Agouti* allele arose de novo after the formation of the Sand Hills. These findings reveal one means by which genetic, developmental, and evolutionary mechanisms can drive rapid adaptation under ecological pressure.

To unravel evolutionary mechanisms in the wild, we must estimate the fitness advantage of adaptive alleles and infer their source, either as new or preexisting variation. In the Sand Hills of Nebraska, deer mice (*Peromyscus maniculatus*) have evolved a dorsal coat that closely matches their local habitat (Fig. 1A and fig. S1). The Sand Hills are a dune field with soil mostly consisting of quartz grains (1, 2) that are lighter in color than the surrounding soils. *P. maniculatus* coat color is correlated with this soil color (3), which is probably due to selection against avian predation (4). Because light-colored mice are conspicuous prey on dark soils (5) and because the Sand Hills are geologically young [dating between the end of the Wisconsin glacial period (10,000 to 15,000 years ago) to within the last 8000 years (6, 7)], the light coat coloration in Sand Hills mice represents a recent adaptation.

Wider hair bands make lighter mice. To a large extent, variation in mammalian pigmentation can be explained by the distribution and relative amounts of brown-black eumelanin and yellow-red pheomelanin pigments in individual hairs (8, 9). Pigment-producing cells (melanocytes) at the base of hair follicles can switch between the production of these pigments during hair growth, resulting in hairs with different banding patterns. Many mammals, including laboratory mice, can have hairs containing a subapical band of pheomelanin on an otherwise eumelanin hair. In *Peromyscus*, this pheomelanin band is markedly wider in Sand Hills mice than in their

darker conspecifics (Fig. 1B). Accordingly, the light Sand Hills phenotype has been dubbed “wideband” (10).

To evaluate the relationship between a mouse’s overall lightness and the size of pheomelanin bands on its dorsal hairs, we compared band width and brightness in five wideband and five wild-type *P. maniculatus* (Fig. 1C). Band width was measured by excising skin plugs from the dorsum and measuring the relative areas of yellow and black pigments in micrographs of the attached hairs (Fig. 1B) (11). We quantified pelt brightness by measuring the percentage of light reflected using a spectrophotometer and found that band width and reflectance were strongly correlated ($R^2 = 0.849$; analysis of variance; $n = 10$ mice, $P = 0.00015$) (Fig. 1C). Thus, the increased width of the subapical pheomelanin band on dorsal hairs is, to a large degree, responsible for the overall lighter color in mice derived from the Sand Hills.

***Agouti* is responsible for the wideband phenotype.** Given its role in producing pheomelanin and its known phenotypic effects, *Agouti* is a strong candidate for the gene causing the wideband phenotype. In *Mus musculus*, knockouts of *Agouti* result in eumelanin (dark-colored) mice, overexpression of *Agouti* results in pheomelanin (light-colored) mice, and light alleles are generally dominant to dark ones (12–14). Consistent with this dominance hierarchy, we found that all offspring from a cross between pure wideband (a^{wb}/a^{wb}) and pure wild-type (a^+/a^+) *P. maniculatus* were phenotypically wideband ($n = 31$ mice). Furthermore, a pulse of *Agouti* expression during hair growth in wild-type *M. musculus* correlates with the production of subapical pheomelanin bands (12, 15). To determine whether the wideband phenotype in *Peromyscus* is caused by *Agouti*, we intercrossed *P. maniculatus* that were heterozygous for nonagouti (a^-) (16), which is a melanic strain resulting from a recessive 125-kb deletion lacking *Agouti* expres-

sion (17). The resulting ratio of wideband:nonagouti phenotypes among the offspring did not differ significantly from 3:1 (χ^2 test; $n = 49$ mice, $P = 0.46$) (table S1), suggesting that the wideband phenotype segregates as a single dominant *Agouti* allele (18). Furthermore, the 125-kb deletion segregated perfectly with coat-color phenotype among the offspring; markers for other pigmentation genes (*attractin* and *tyrosinase-related protein 1*) did not (table S1). Finally, no sequence differences were observed between wild-type, wideband, and nonagouti *P. maniculatus* in the *melanocortin-1 receptor* (*Mclr*)-coding region ($n = 7$ a^{wb}/a^- ; $n = 2$ a^-/a^- , and $n = 1$ a^+/a^+ , respectively).

Agouti mRNA levels in wideband/nonagouti *P. maniculatus* were significantly higher than in wild-type/nonagouti mice (a^{wb}/a^- versus a^+/a^- t test, $n = 8$ mice, $P = 0.0069$, one-tailed) (Fig. 2A). This expression difference persisted when abundance of both alleles was measured separately in a^{wb}/a^+ heterozygotes (a^{wb} versus a^+ t test; $n = 8$ alleles, $P = 0.00031$, one-tailed) (Fig. 2A), demonstrating that one or more mutations acting in *cis* lead to an increase in *Agouti* expression (19). These data indicate that a *cis*-acting mutation (or mutations) in, or linked to, the *Agouti* gene is responsible for the wideband phenotype.

We also measured *Agouti* mRNA levels over a synchronized hair cycle (as hairs first emerge simultaneously in newborn mice) in neonatal wideband and wild-type *P. maniculatus*. Because *Agouti* initiates the production of the pheomelanin hair band during a pulse of expression occurring postnatally from day 3 to 7 in *Mus musculus* (15), we measured *Agouti* expression between birth and day 8 in wideband and wild-type *P. maniculatus* (Fig. 2B). Between days 1 and 5 inclusively, *Agouti* mRNA was significantly more abundant in wideband mice than in wild-type (t tests; $n = 7$ to 9 mice for each time point, $P < 0.05$, one-tailed) (Fig. 2B). Although *Agouti* expression peaks at day 4 in both wideband and wild-type mice, in wideband mice expression peaks above wild-type levels by day 1 and remains at or above this level until day 7 (Fig. 2B). Thus, the pulse of *Agouti* expression during hair growth is both longer and higher in wideband postnatal mice, suggesting that increased *Agouti* expression is the underlying cause of the wideband phenotype in *P. maniculatus*.

Agouti produces two transcriptional isoforms, each with a different expression profile (15, 20). The hair cycle-specific isoform that produces the hair band in *Mus* contains untranslated exons 1B or 1C and is expressed in a timed pulse during hair growth (15). We sequenced exons 1B, 1C, 2, 3, and 4 in four wideband and four wild-type *P. maniculatus* and compared these with a *P. maniculatus rufinus* individual with a wild-type phenotype, as an outgroup. Within the transcript expressed in the hair cycle, we identified 20 nucleotide differences between wideband and wild-type mice, 11 of which were derived relative to the outgroup in wideband mice (Fig. 3A). Seven

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differences (four derived) were observed within the coding region, of which two resulted in amino acid changes in wideband mice, a conservative amino acid substitution (Arg37Lys) and a serine deletion (residue 48), both in exon 2. All polymorphisms were perfectly associated with the wideband phenotype in laboratory populations of *Peromyscus*.

Wideband allele frequency in natural populations. To test whether any of these *Agouti* polymorphisms were associated with the wideband phenotype in nature, we captured 91 individuals from two phenotypically variable populations near the edge of the Sand Hills (Fig. 1A and table S2). We collected phenotypic (band width and brightness) and genotypic (*Agouti* hair-cycle transcript sequence and alleles at seven microsatellite markers) data for each individual. We pooled individuals from the two locations for association tests because we did not detect differences in color among the populations (brightness, *t* test; $n = 91$ mice, $P = 0.90$, two-tailed). Moreover, although the program STRUCTURE (21, 22) identified two genetic clusters ($K = 2$), which corresponded to geography [Fisher's exact test; $n = 85$ mice, $P = 0.0039$; mean fixation index (F_{ST}) across seven microsatellite loci was 0.020 ± 0.003 SEM], there was no correlation with phenotype (brightness, *t* test; $n = 91$ mice, $P = 0.89$, two-tailed). These results suggest that mice do not mate assortatively on the basis of coat color and that dark-colored mice are not recent, genetically distinct migrants. Therefore, we hypothesized that light and dark mice interbreed with ample opportunity for recombination between the wideband and wild-type alleles.

Because band width and brightness were more variable in Sand Hills mice than in the laboratory

populations (fig. S2, A and B), we used quantitative measures of both band width and brightness, rather than discrete categories, for association tests. At the *Agouti* locus, most of the hair-cycle transcript polymorphisms identified in the lab were also variable among Sand Hills mice (Fig. 3A). Of these candidate polymorphisms, only the serine deletion in exon 2 explained variation in band width (13.2% of the observed variation) and in overall brightness (8.8% of variation) after correction for multiple comparisons (Fig. 3A) and was consistent with observed dominance patterns from laboratory crosses. Specifically, individuals with one or more copies of the deletion ($a^{\Delta Ser}/a^+$) were significantly lighter than those without (a^+/a^+) (*t* tests;

$n = 91$ mice; band width, $P = 0.00020$, one-tailed; brightness, $P = 0.0022$, one-tailed), whereas heterozygotes ($a^{\Delta Ser}/a^+$) and homozygotes ($a^{\Delta Ser}/a^{\Delta Ser}$) were phenotypically indistinguishable (*t* tests; $n = 62$ mice; band width, $P = 0.81$, two-tailed; brightness, $P = 0.39$, two-tailed).

This serine deletion occurs in an evolutionarily conserved region of the *Agouti* protein that interacts with Attractin, an accessory receptor thought to facilitate *Agouti*'s role in pigment-switching (23). It is therefore possible that the serine deletion is directly responsible for the wideband phenotype. Alternatively, the deletion could be in linkage disequilibrium (LD) with the causal mutation (or mutations). We thus examined pat-

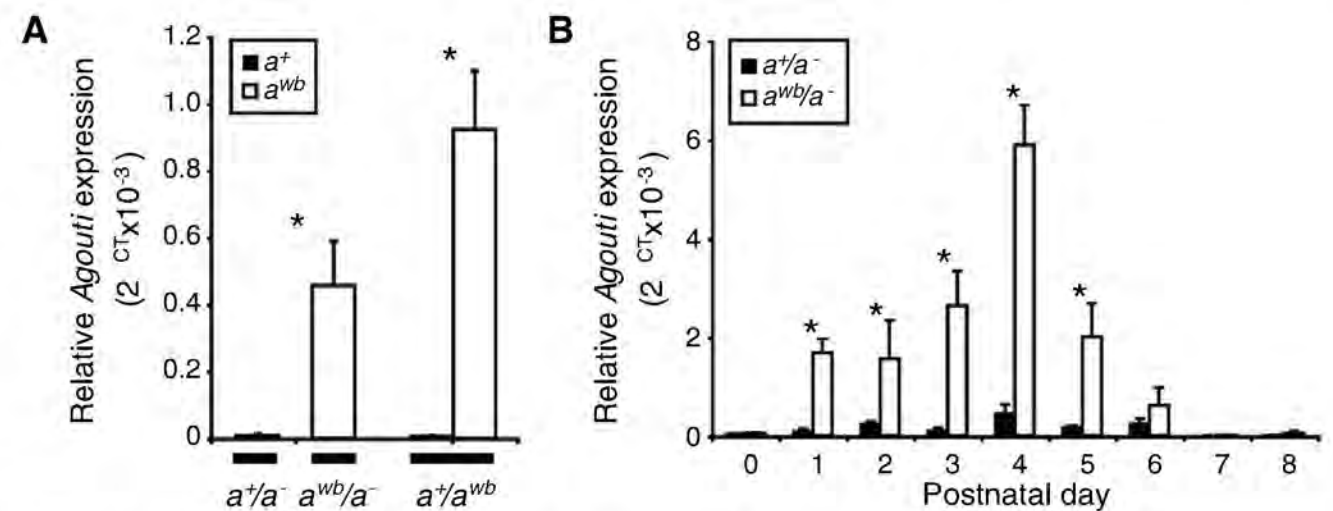
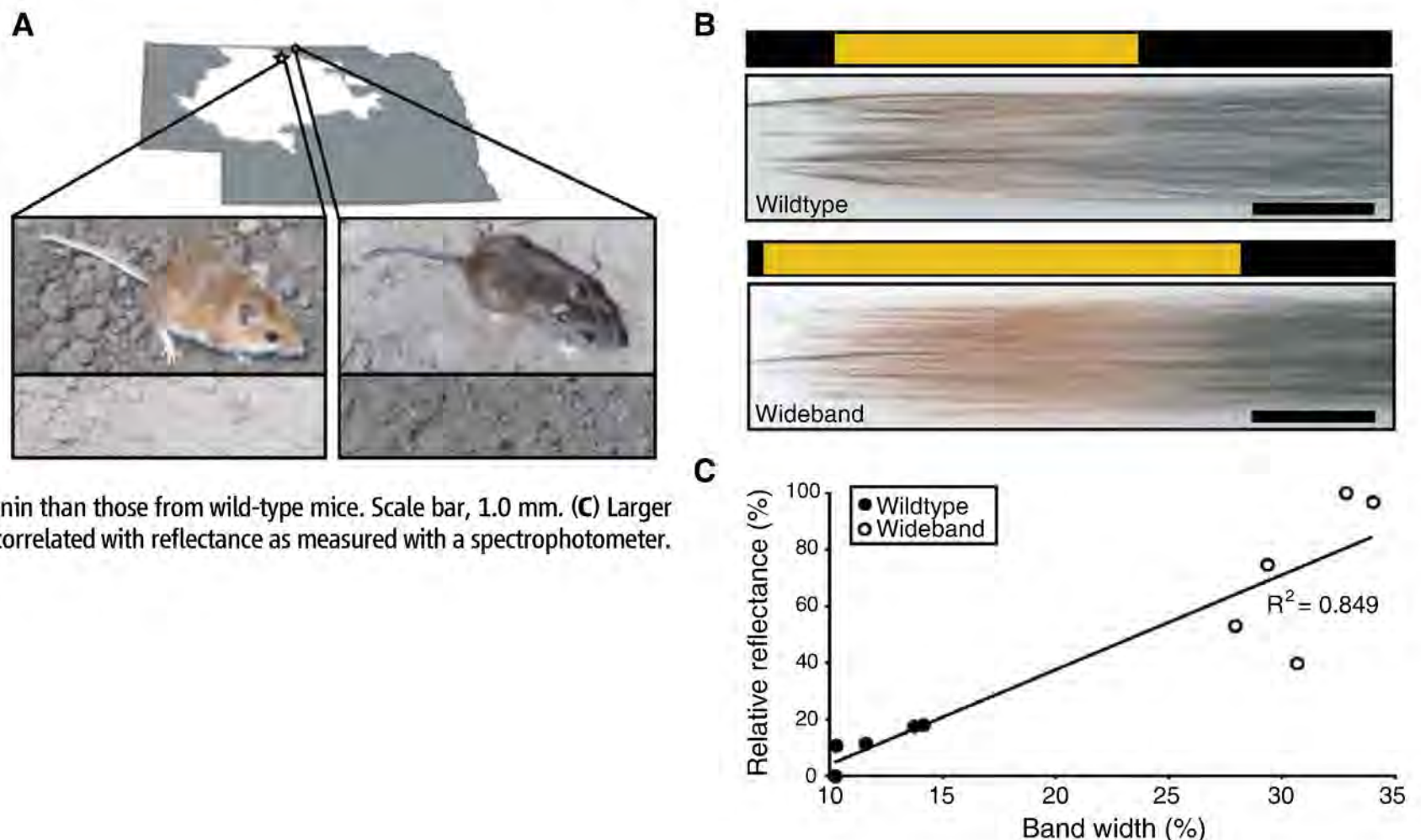


Fig. 2. A *cis*-acting mutation (or mutations) increases *Agouti* expression and causes the wideband phenotype. (A) Quantitative real-time polymerase chain reaction assays show that *P. maniculatus* with the wideband allele (a^{wb}) express *Agouti* at a higher level than do those with the wild-type allele (a^+). In heterozygotes (a^+/a^{wb}), the a^{wb} allele is expressed at a higher level than the a^+ allele, suggesting that the causal mutation (or mutations) acts in *cis*. (B) Expression of *Agouti* transcript is significantly higher in wideband mice from postnatal day 1 to day 5 (asterisks denote significance at $P < 0.05$, *t* test, $n = 3$ to 6 mice for each genotype and time point). *Agouti* expression was measured relative to β -actin; data are mean $\pm$ SEM.

Fig. 1. Soil and mice are brighter on the Sand Hills because of band width. (A) Approximate location of Sand Hills within Nebraska. Typical soil (bottom) and mouse (top) colors for populations sampled on (star) and off (circle) the Sand Hills. Mice are shown on contrasting soil backgrounds. (B) Hairs from the dorsum of wideband mice have a wider band of pheomelanin than those from wild-type mice. Scale bar, 1.0 mm. (C) Larger band area is significantly correlated with reflectance as measured with a spectrophotometer.



terns of LD upstream and downstream of the deletion. We observed significant LD across the entire 1-kb region flanking exon 2 and found that LD rapidly decays downstream (3'), but not upstream (5'), of the deletion (Fig. 3B) when we examined high-frequency polymorphisms (the minor allele frequency was >10%). Additionally, LD persisted as far as 19 kb upstream of the deletion but not past 600 bp downstream (table S3). On the basis of these results, we cannot determine whether the serine deletion, a linked mutation, or both cause wide bands and light coats. Given the rapid decay of LD downstream of the deletion, the observed extended upstream LD may be due to epistatic selection on multiple coadapted mutations (24, 25). Nonetheless, the haplotype containing the serine deletion (hereafter "wideband haplotype" or "wideband allele") explains a substantial

amount of ecologically relevant phenotypic variation.

Selection on the wideband haplotype. To compare putatively adaptive (wideband or $a^{\Delta Ser}$) and neutral (wild-type or a^+) haplotypes, we examined nucleotide variation in the two haplotype classes. A significant reduction in variation, which was consistent with patterns expected under a selective sweep, was observed in the wideband haplotype as compared with that in the wild-type haplotype [number of segregating sites (S) = 52 and nucleotide diversity (π) = 0.0081 for n = 80 wideband versus S = 80 and π = 0.0133 for n = 102 wild type] (Fig. 4A). We also found that the shape of the full site frequency spectra (SFS) for the haplotype classes differed between wild-type and wideband haplotypes (fig. S3). The wild-type haplotype was not distinguishable from that predicted under a neutral equilibrium model,

whereas the wideband haplotype showed a strong skew in the SFS, matching a hitchhiking model (a U-shaped SFS).

To distinguish between selective and neutral models, we performed a composite likelihood ratio (CLR) test (26) and separately evaluated the evidence for selection on the wideband and wild-type haplotypes [following the method in (27)]. A significant signature of positive selection was observed for the wideband haplotype (CLR test; P = 0.012) but not on the remaining chromosomes (CLR test; P = 0.123). We then used the goodness-of-fit (GOF) test (28) on the wideband haplotype and found that the hitchhiking model could not be rejected (GOF P = 0.813). Finally, we employed the ω_{max} statistic (29, 30) and found that the LD patterns across the wideband haplotype were significantly different from those expected under neutrality (ω_{max} ; P = 0.041), whereas LD patterns for the wild-type haplotype classes were not (ω_{max} ; P = 0.345). Together, these results demonstrate that selection is probably acting on the wideband *Agouti* haplotype.

Additionally, these analyses identified a location at or near the serine deletion that is the nucleotide site that maximizes the significance of the CLR and ω test statistics (26, 29) and thus is the most likely target of selection in our data (Fig. 3B). Because patterns of variation around a selected site may only be expected to be symmetric on average, but not in any individual realization (26), the noted patterns of asymmetric LD surrounding the deletion are consistent with our conclusion (Fig. 3B).

Strength and timing of selection. To estimate the selection coefficient acting on the wideband haplotype, we obtained maximum likelihood estimates of $\alpha = 2Ns$, where N is the effective population size, via maximization of the composite likelihood function (26) and found an estimate of $\alpha = 112$. Assuming N = 10,000 (31), this corresponds to an estimated selection coefficient of $s = 0.0056$. Using a parametric bootstrap (32), we obtained a 95% confidence interval (CI) (constructed with the percentile method) that spans from $\alpha = 56$ to $\alpha = 207$ ($s = 0.0028$ to 0.0104). Based on owl predation experiments (4) and the observed *Agouti* allele frequencies, we estimated that when on light soil wideband mice have a selective advantage $s = 0.102$ as compared with darker mice. This is certainly an overestimate of selection, given that predation rates were most likely artificially inflated in the enclosed arena tested (4) and that other loci probably contribute to the Sand Hills mouse phenotype. Nevertheless, both population genetic data and predation experiments suggest that selection for light color is strong, and our estimates fall within the range of selection coefficients for other color polymorphisms, including in beach mice (33), pocket mice (5), ladybirds (34), and land snails (35).

Based on our estimates of the selection strength acting on the wideband allele, we inferred the timing of selection on this allele. From

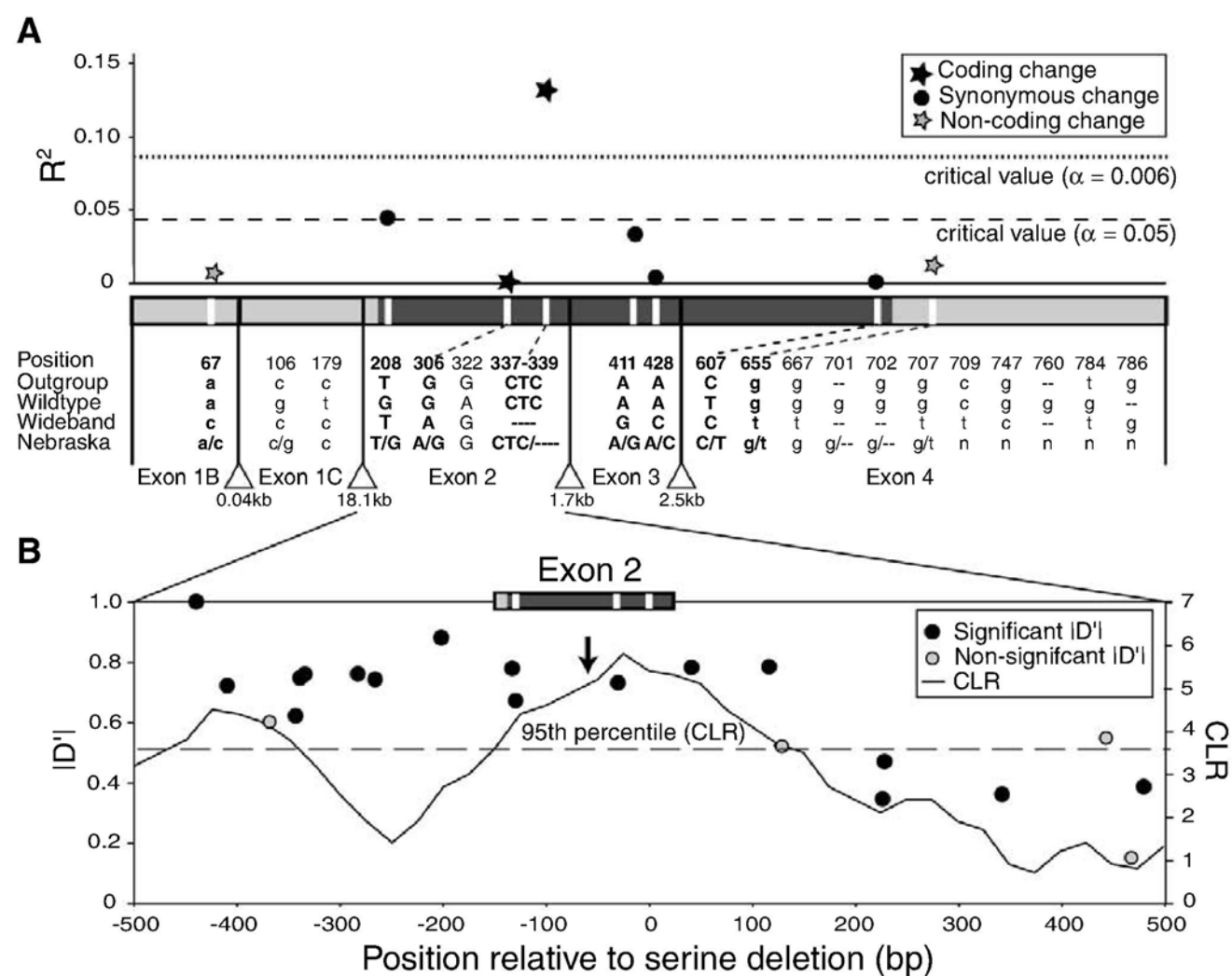


Fig. 3. A coding deletion in exon 2 is associated with band width and sweep-like patterns of genetic variation. **(A)** The correlation (R^2) between genotype and phenotype (band width) in a natural population. Hair-cycle transcript exons (x axis) are drawn to scale and coded as follows: translated regions are in dark gray and uppercase; untranslated regions are in light gray and lowercase; and introns are indicated by triangles. Significance thresholds are before (α = 0.05) and after (α = 0.006) Bonferroni correction. Listed are all sites that differed between wideband and wild-type mice, along with an outgroup (*P. maniculatus rufinus*), and the consensus sequence for 91 wild-caught individuals (Nebraska). R^2 is plotted for eight polymorphic sites [minor allele frequency (MAF) > 2%] in the Nebraska population (white vertical bars and bolded letters). Length heterozygosity resulted in some missing data; these sites were excluded from analysis. A derived amino acid deletion in exon 2 explains 13% of the phenotypic variation in Nebraska mice. **(B)** LD (left axis) and signature of selection (right axis) across the exon 2 flanking region. Position relative to exon 2 (top) or to the exon 2 serine deletion (bottom) is given. Each point is pairwise LD (ID'I, left axis) between a common polymorphism (MAF > 10%) and the serine deletion; black points are significant via χ^2 tests after Bonferroni correction. Also given is the CLR (right axis) as a function of position (solid line) for the wideband haplotype. The dotted line corresponds to the 95th percentile of the CLR (3.62); the arrow indicates the position that maximizes the ω statistic (ω_{max}). SFS- and LD-based analyses both point to the serine deletion as the putative target of selection.

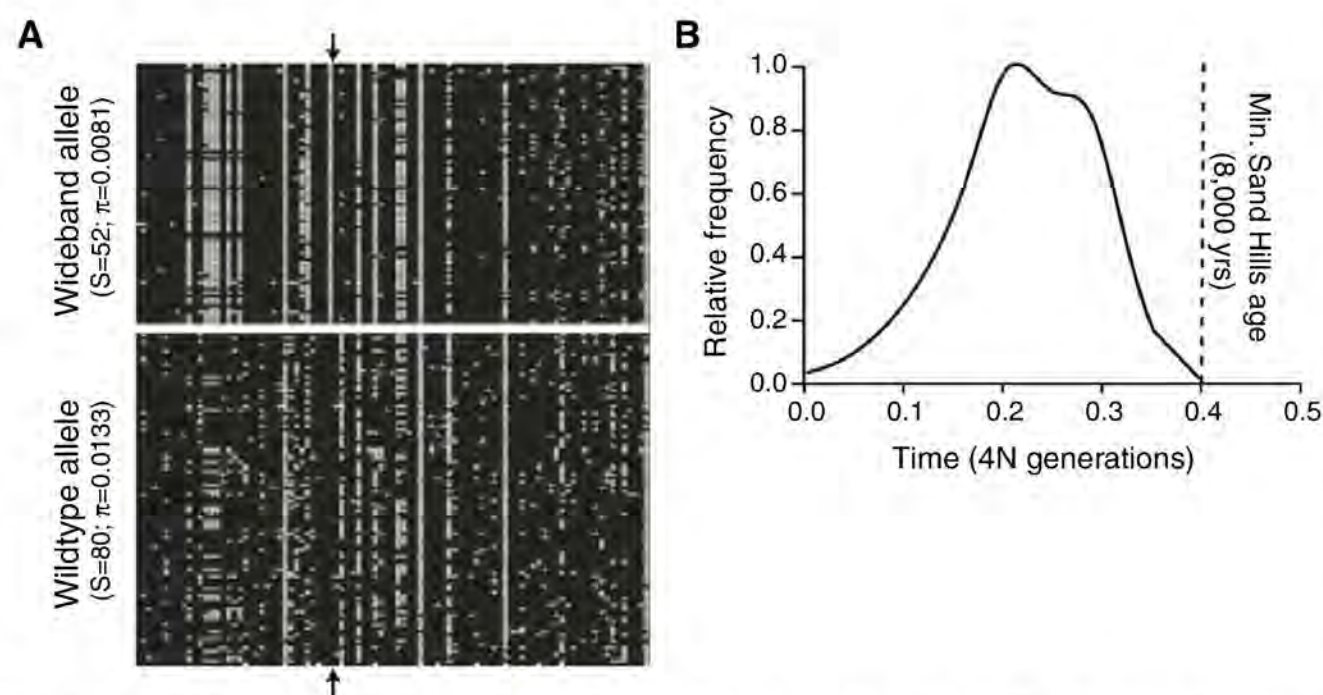


Fig. 4. The wideband mutation arose de novo after formation of the Sand Hills. **(A)** Variation in exon 2 flanking region for wideband ($a^{\Delta Ser}$, $n = 80$ alleles) and wild-type (a^+ , $n = 102$ alleles) alleles. Rows are observed haplotypes; columns are variable nucleotide sites (black indicates ancestral, white indicates derived, and the outgroup is *P. maniculatus rufinus*). Arrows indicate the derived serine deletion. S and π are given for both haplotypes. Reduced variation among wideband alleles matches the pattern expected under selection on a de novo mutation. **(B)** Posterior probability distribution for the age (in $4N$ generations) of the beneficial wideband allele ($a^{\Delta Ser}$), and minimum age of the Sand Hills (8000 years, or 0.4 $4N$ generations assuming $N = 10,000$ and 2 generations per year).

our 95% CI of s and calculation of $\sim 2\ln(2N)/s$ (36), we predict that the wideband allele should reach fixation (100% frequency) in 0.05 to 0.18 $4N$ generations. These estimates were also similar to those obtained with a Bayesian approach (37), in which we found that over 99.9% of the posterior probability density falls on $0.5 \geq T$, $\sim 85.4\%$ on $0.25 \geq T$, and $\sim 3.7\%$ on $0.01 \geq T$, where T is the age of the beneficial allele in $4N$ generations (Fig. 4B). Given that the estimated age of the Sand Hills is 8000 to 10,000 years (or ~ 0.4 to 0.5 $4N$ generations), our estimates suggest that this allele arose and underwent an incomplete sweep sometime after the formation of the Sand Hills.

Multiple lines of evidence suggest that the wideband allele arose de novo and was not a preexisting allele. First, the haplotype carrying the deletion has greatly reduced variation relative to the wild type (Fig. 4A), which is inconsistent with a model in which the causative mutation was neutrally segregating in the population before any selective pressure (38). Second, the U-shaped SFS is most consistent with a model in which selection acts on a newly arising mutation (fig. S3). If the beneficial mutation existed on multiple haplotypes before the selective pressure, we would expect to see an excess of intermediate frequency mutations, which was not observed (38). Finally, the posterior probability density of the allele age falls entirely within the estimated age of the Sand Hills (Fig. 4B).

Taken together, our results demonstrate that variation at the *Agouti* locus is responsible for adaptive coloration in deer mice living on the Nebraska Sand Hills. Although it is clear that a derived increase in *Agouti* expression leads to

wider hair bands and lighter camouflaging color, whether and by which mechanism an amino acid deletion ($a^{\Delta Ser}$) leads to a change in gene expression and ultimately phenotypic evolution is still unknown. From our estimates of the strength and timing of selection acting on this adaptive allele, we conclude that the wideband *Agouti* allele arose de novo after the colonization of a novel selective environment, which is counter to recent studies demonstrating adaptation arising from standing genetic variation (39–41). This suggests that rapid adaptive change—such as the recent evolution of cryptic coloration in Sand Hill mice—need not always rely on preexisting genetic variation.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5944/1095/DC1
Materials and Methods

Figs. S1 to S3

Tables S1 to S5

References

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Spectroscopic Fingerprint of Phase-Incoherent Superconductivity in the Underdoped $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$

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A possible explanation for the existence of the cuprate “pseudogap” state is that it is a d-wave superconductor without quantum phase rigidity. Transport and thermodynamic studies provide compelling evidence that supports this proposal, but few spectroscopic explorations of it have been made. One spectroscopic signature of d-wave superconductivity is the particle-hole symmetric “octet” of dispersive Bogoliubov quasiparticle interference modulations. Here we report on this octet’s evolution from low temperatures to well into the underdoped pseudogap regime. No pronounced changes occur in the octet phenomenology at the superconductor’s critical temperature T_c , and it survives up to at least temperature $T \sim 1.5 T_c$. In this pseudogap regime, we observe the detailed phenomenology that was theoretically predicted for quasiparticle interference in a phase-incoherent d-wave superconductor. Thus, our results not only provide spectroscopic evidence to confirm and extend the transport and thermodynamics studies, but they also open the way for spectroscopic explorations of phase fluctuation rates, their effects on the Fermi arc, and the fundamental source of the phase fluctuations that suppress superconductivity in underdoped cuprates.

Superconductivity in the hole-doped CuO_2 Mott insulators is unique by virtue of the convergence of three phenomena. First, the superconductivity is quasi-two-dimensional, because it occurs primarily in the CuO_2 planes. Second, presumably because of correlations, the superfluid density is very low and increases from zero approximately linearly with the hole density p . Third, because it is a d-wave system, the superconducting energy gap $\Delta(\vec{k})$ exhibits four k -space nodes where zero-energy excitations exist. This unique combination means that fluctuations of the quantum phase $\varphi(\vec{r}, t)$, where $\vec{r}$ is the position vector and t is time, of the superconducting order parameter $\Psi = \Delta e^{i\varphi(\vec{r}, t)}$ should have profound effects on cuprate superconductivity at low hole density (1–6). One might then expect to observe a sequence of three different temperature scales. The highest characteristic temperature would occur at the mean-field scale, T_{MF} , where local pairing begins. The next, T_ϕ , is where strong diamagnetic fluctuations would set in. The lowest is the true superconducting critical temperature, T_c , where phase rigidity would appear in the presence of whatever combination of ther-

mal and quantum fluctuations (7–12) actually exists in underdoped cuprates.

A schematic of the hole-doped cuprate phase diagram is shown in fig. S1A; within the gray shaded pseudogap regime, the approximate region where transport and thermodynamic evidence for phase-incoherent superconductivity has been detected (13–18) is shaded light blue. The techniques used include terahertz transport studies (13), the Nernst effect in thermal transport (14, 15), torque-magnetometry measurements of diamagnetism (16), field dependence of the diamagnetism (17), and zero-bias conductance enhancement in tunnel junctions linking pseudogapped to superconducting samples (18). But the regions designated as containing phase-incoherent superconductivity differ markedly between these studies. Moreover, it has been proposed recently that much of the Nernst signature attributed to phase fluctuations may be due to the appearance of stripes (19). Thus, a direct spectroscopic fingerprint of phase-incoherent d-wave superconductivity could help to identify the precise regions dominated by phase fluctuations. Perhaps more importantly, the associated spectroscopic phenomena might be used to quantify phase fluctuation rates and to discriminate between different quantum fluctuation sources (7–12) causing the loss of phase rigidity, and thus suppression of high-temperature superconductivity, in underdoped cuprates.

Spectroscopic imaging scanning tunneling microscopy (SI-STM) provides one approach to search for a spectroscopic fingerprint of phase-incoherent d-wave superconductivity. With this technique, the local density of states $N(\vec{r}, E)$

(where E is energy), the two branches of the Bogoliubov excitation spectrum $\vec{k}_B(\pm E)$, and the superconducting energy gap magnitude $\pm|\Delta(\vec{k})|$ for both filled and empty states can be determined in a single experiment (20–23). This is because a characteristic form of quasiparticle interference (QPI) occurs in cuprate superconductors where the Bogoliubov quasiparticle dispersion $E(\vec{k})$ has banana-shaped constant energy contours (20–23), as shown schematically in fig. S1B. In theory, the k -space locations of the Bogoliubov band minima and maxima, $\vec{k}_B(\pm E)$, coincide with the maxima in the joint density-of-states at the eight tips of these “bananas.” Elastic scattering between these eight regions $\vec{k}_j(\pm E)$ ($j = 1, 2, \dots, 8$) produces real-space interference modulations in $N(\vec{r}, E)$ that are characterized by two sets of seven dispersive wave vectors $\vec{q}_i(\pm E)$ ($i = 1, 2, \dots, 7$), where $\vec{q}_i(+E) = \vec{q}_i(-E)$ (fig. S1B). This is referred to as the octet model of cuprate QPI (20–23) and, for $T \ll T_c$, it is a definitive signature of d-wave superconductivity. When these $\vec{q}_i(E)$ are measured from the Fourier transform of modulations in the differential tunneling conductance maps, $g(\vec{r}, E) \equiv dI/dV(\vec{r}, E)$, where I is the tunnel current and V is the tunnel-junction bias voltage, the Bogoliubov bands $\vec{k}_B(\pm E)$ and the superconductor’s energy gap structure $\pm|\Delta(\vec{k})|$ can be determined (20–23). This technique is unique in that the measured gap $\pm|\Delta(\vec{k})|$ is definitely that of the superconductor and, because the technique uses visualization of interference patterns, it automatically identifies k -space regions having coherent quasiparticles.

A possible signature of phase-incoherent d-wave superconductivity in the pseudogap regime could be the continued existence of this QPI octet phenomenology. This is because, if the quantum phase $\varphi(\vec{r}, t)$ of $\Psi = \Delta e^{i\varphi(\vec{r}, t)}$ is fluctuating in space and time, then the energy gap magnitude $\pm|\Delta(\vec{k})|$ could still remain largely unchanged so that the particle-hole-symmetric octet of high joint-density-of-states regions generating the QPI would continue to exist. Because an ungapped Fermi arc appears above T_c in underdoped cuprates (24), it is only the remaining gapped regions beyond the tips of the arc that would be available to support any such pseudogap QPI. Indeed, detailed theoretical studies of the QPI phenomenology that would be expected if the pseudogap regime is a phase-incoherent d-wave superconductor bear out this simple picture (25–27). Among their predictions is the existence of a particle-hole-symmetric octet of dispersive QPI modulations, but perhaps the octet might emerge from a k -space region that is different from that in the superconducting phase.

However, as revealed by the pioneering SI-STM studies at temperatures above T_c (28), the detection of the complete octet of dispersive QPI modulations in the pseudogap regime presents severe technical challenges. Because the basic observables are the particle-hole-symmetric dispersions $\vec{q}_i(+E) = \vec{q}_i(-E)$ for the seven wave

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vectors $\vec{q}_i$, the primary objectives are to achieve high precision in simultaneous measurements of all seven $|\vec{q}_i|$ and of E within the pseudogap regime. Because the uncertainty in energy of a tunneling electron grows rapidly with temperature (exceeding 30 meV at 100 K), precision in the measurement of E requires the lowest possible temperatures. We therefore chose to reduce T_c of our $\text{Bi}_2\text{Sr}_2\text{Ca}_{0.8}\text{Dy}_{0.2}\text{Cu}_2\text{O}_{8+\delta}$ sample to 37 ± 3 K by strong underdoping so that the pseudogap regime could be entered at low temperatures (fig. S1A, inset). Second, adequate $|\vec{q}_i|$ precision for $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$ QPI requires fields of view (FOVs) exceeding $45 \times 45 \text{ nm}^2$, and any smaller FOV will unavoidably create the erroneous impression of nondispersive modulations (21). Thus, all studies herein were made on an FOV exceeding $45 \times 45 \text{ nm}^2$ (for example, fig. S2). Even more challenging (as we show below) is that the intensities for some $\vec{q}_i(E)$ modulations in the pseudogap regime diminish by more than a factor of 10 compared with the superconducting state, so that greatly increased signal-to-noise ratios for all $g(\vec{r}, E)$ measurements are required. To achieve this increased ratio, we acquired each $g(\vec{r}, E)$ map for up to 10 days by using a specially designed low-noise preamplifier, a highly drift- and temperature-stabilized STM system, and a replicating single-atom tip preparation technique. Finally, because the truly nondispersive electronic structure of the high-energy states (23, 29) is projected onto the low-energy $g(\vec{r}, E)$ by the systematic junction formation error (22, 23), the resulting spurious appearance of nondispersive signals at low bias is avoided here by the use of the relation $Z(\vec{r}, E) \equiv g(\vec{r}, +E)/g(\vec{r}, -E)$ for all QPI analyses (22). Using $Z(\vec{r}, E)$ and $Z(\vec{q}, E)$ also has the advantage that it automatically extracts particle-hole symmetric $\vec{q}_i(+E) = \vec{q}_i(-E)$ QPI modulations (22, 23).

By combining all these capabilities in a specially designed, variable-temperature SI-STM system, we were able to study the temperature evolution of the octet of QPI modulations in $Z(\vec{q}, E)$ from the superconducting phase into the pseudogap regime. The octet of interference modulations was studied at temperatures $T = 4.5, 15, 30, 37, 45$, and 55 K, thereby entering well into the strongly underdoped pseudogap regime (fig. S1A). We measured the $g(\vec{r}, E)$ images with subatomic resolution (fig. S2) on a single $\text{Bi}_2\text{Sr}_2\text{Ca}_{0.8}\text{Dy}_{0.2}\text{Cu}_2\text{O}_{8+\delta}$ sample with $p = 7 \pm 1\%$. This data set consists of $\sim 5 \times 10^5$ atomically resolved and registered tunneling spectra and is described in the supporting online material (SOM) for every temperature T , in terms of a comprehensive set of $Z(\vec{r}, E)$ images (fig. S3) and the related $Z(\vec{q}, E)$ movies (movies S1 to S6).

Representative $Z(\vec{q}, E)$ for six temperatures are shown in Fig. 1. Several important observations can be made from these data. First, the set of $\vec{q}_i(E)$ ($i = 1, 2, \dots, 7$) that is characteristic of the superconducting octet model is preserved unchanged upon passing above T_c and exists up to at least $T \sim 1.5T_c$ [compare, for example, (A) and

(U)]. This result demonstrates that the QPI octet phenomenology exists in the pseudogap regime. Second, the existence of such interference patterns indicates that coherent wavelike quantum states occur in some parts of k -space in the pseudogap regime. Third, the octet of QPI wave vectors are quantitatively different at different temperatures, indicating that they are generated by different regions of k -space, and thus that $\Delta(\vec{k})$ is evolving with temperature. Fourth, some, but not all, QPI modulation intensities become far weaker in the pseudogap regime.

The measured values of $|\vec{q}_1(E)|$, $|\vec{q}_5(E)|$, and $|\vec{q}_7(E)|$ at each temperature shown in Fig. 2 reveal that the modulations that are dispersive in the superconducting phase (20–23) remain dispersive into the pseudogap regime. This is equally true for every measured octet wave vector (see fig. S4 and movies S1 to S6). Moreover, if d-wave energy gaps without particle-hole symmetry were generating these pseudogap QPI effects, instead of the 16 pairs of modulation wave vectors observed here, $Z(\vec{q}, E)$ should exhibit 32 pairs because then, $\vec{q}_i(+E) \neq \vec{q}_i(-E)$. These obser-

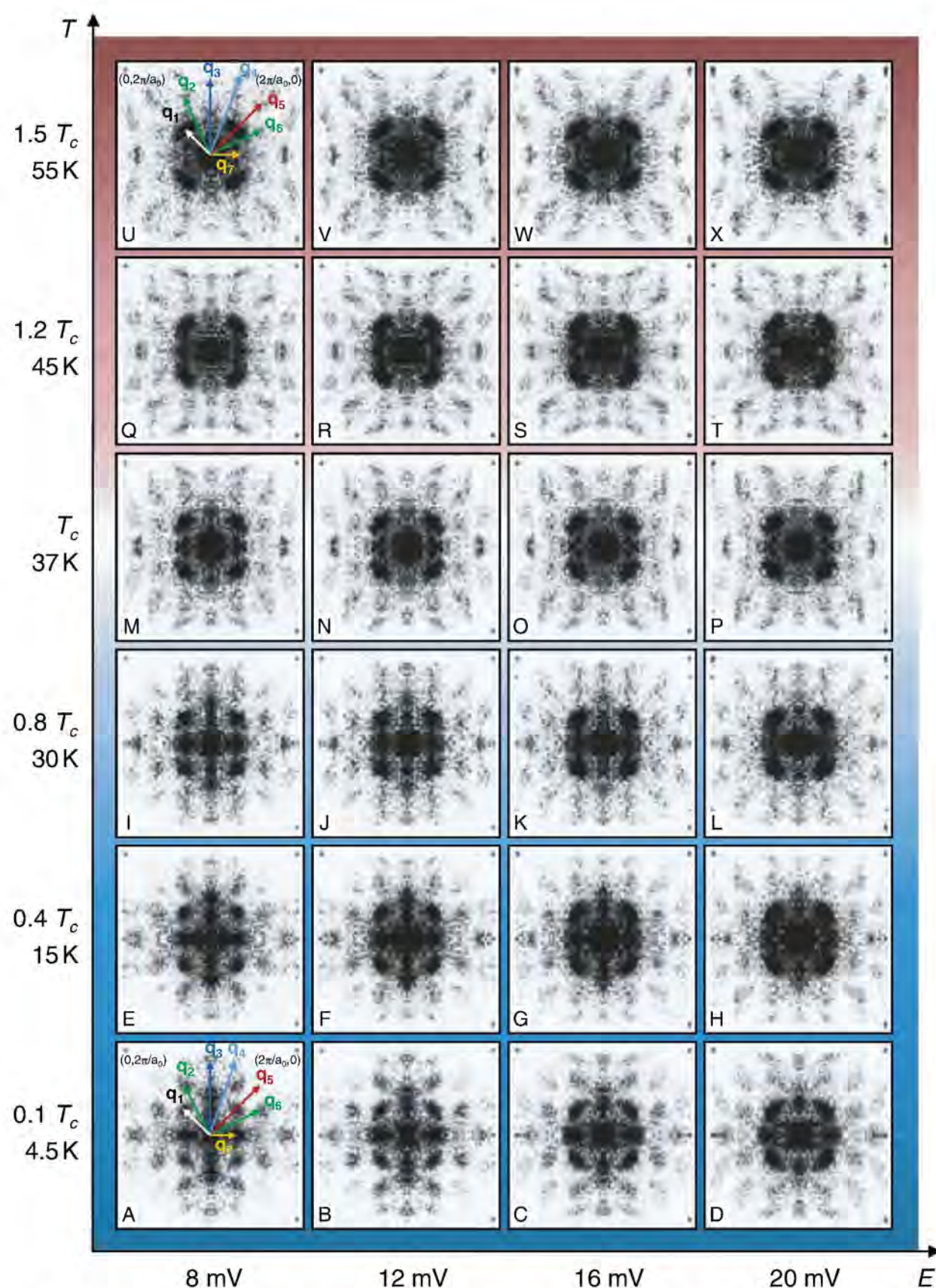


Fig. 1. (A to X) Differential conductance map $g(\vec{r}, E) = dI/dV(\vec{r}, E = eV)$ were obtained on the same sample in an atomically resolved and registered FOV $> 45 \times 45 \text{ nm}^2$ at six temperatures (fig. S2). Each panel shown is the Fourier transform $Z(\vec{q}, E)$ of $Z(\vec{r}, E) \equiv g(\vec{r}, +E)/g(\vec{r}, -E)$ for a given energy and temperature (fig. S3). The QPI signals evolve dispersively with energy along the horizontal energy axis. The temperature dependence of QPI for a given energy evolves along the vertical axis. The octet-model set of QPI wave vectors is observed for every E and T (see movies S1 to S6) as seen, for example, by comparing (A) and (U), each of which has the labeled octet vectors. Within the basic octet QPI phenomenology, there is no particular indication in these data of where the superconducting transport T_c occurs.

vations are important for discrimination between any static electronic ordered state having non-dispersive modulations at an ordering wave vector $\vec{Q}$ and the dispersive k -space eigenstates of a phase-incoherent d-wave superconductor. Because we find that for $|E| < 35$ meV, all detected $\vec{q}_i(E)$ in the pseudogap regime are dispersive and particle-hole symmetric (Fig. 2 and fig. S4) and, moreover, that the dispersions are internally consistent with the octet model (fig. S4), we conclude that these low-energy modulations

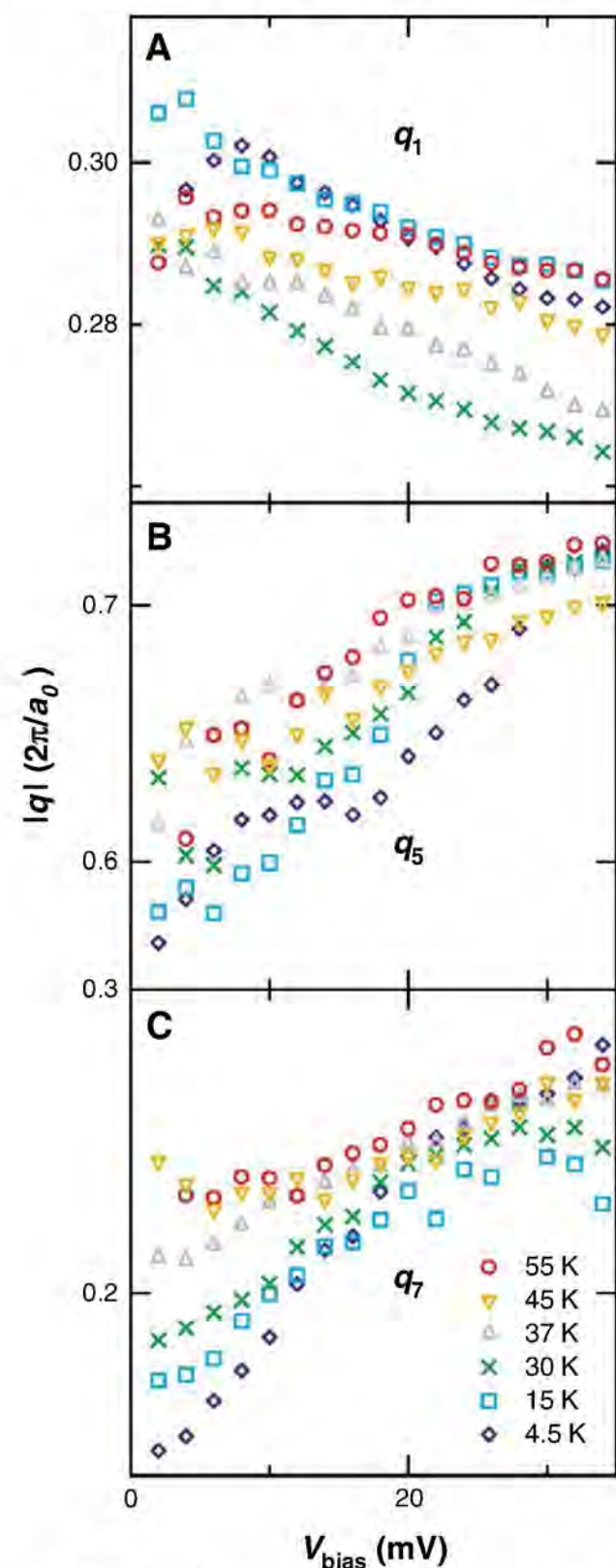


Fig. 2. (A to C) Temperature dependence of the length of representative q -vectors— $|\vec{q}_1(E)|$, $|\vec{q}_5(E)|$, and $|\vec{q}_7(E)|$ —as a function of energy. We show here and in fig. S4 and movies S1 to S6 that octet modulation vectors $\vec{q}_1$, $\vec{q}_2$, $\vec{q}_4$, $\vec{q}_5$, $\vec{q}_6$, and $\vec{q}_7$ are manifestly dispersive at all temperatures studied, with the gradual changes in length as temperature is increased (fig. S3). Wave vector $\vec{q}_3$ has been difficult to observe at all energies and so is not used in the QPI analysis. Wave vector $\vec{q}_5$ is easily detected and is dispersive as expected; however, it contains a second-order contribution and so is not used in the determination of $\pm|\Delta(\vec{k})|$.

in $Z(\vec{q}, E)$ do not represent the signature of any static, electronic ordered state. However, the true nondispersive excitations of underdoped cuprates (23, 29), which occur at the pseudogap energy scale (near $E \sim \pm 120$ meV in this sample) and break translational and rotational symmetry locally, remain completely unaltered upon the transition through T_c into the pseudogap regime (fig. S7).

By requiring octet-model internal consistency (20–23) in the dispersions of all measured $\vec{q}_i(E)$ for all energies and all temperatures (fig. S4), we can determine $\vec{k}_B(\pm E)$ and $|\Delta(\vec{k})|$ as a function of T . The results shown in Fig. 3A (and figs. S5 and S6) indicate that, even at the lowest temperatures, the locus of scattering $\vec{k}_B(E)$ exhibits the ungapped arc previously reported in (22, 23, 30). Beyond the tips of this arc are the gapped regions that exhibit particle-hole-symmetric QPI, which then disappears at the line connecting $(\pi/a_0, 0)$ and $(0, \pi/a_0)$ (23). Beyond that line, the electronic excitations to the pseudogap energy scale exhibit locally broken translational and rotational symmetries in r -space (23, 29). Figure 3A shows the

energy gap $|\Delta(\vec{k})|$ measured using the standard octet analysis (20–23) as a function of temperature. We see that, whereas the measured $\vec{k}_B(E)$ are on the same contour in k -space at all T (fig. S5), the ungapped arc length grows slowly with increasing T and the gapped regions exhibiting octet QPI thereby become more constricted in k -space (fig. S6). Figure 3B shows that the ungapped arc length is a monotonically increasing function of temperature (figs. S6 and S8). Moreover, the simultaneously measured zero-bias conductance shown in Fig. 3B (which is proportional to the density of states at the Fermi energy shown in Fig. 3C) also exhibits a high value at lowest temperatures and increases linearly with T . This result provides additional evidence for an ungapped zero-temperature arc that lengthens linearly with T . If such an ungapped arc in the superconducting state can be generated either by scattering (31, 32) or by non-thermally generated phase fluctuations (6), then these results appear to be consistent with the linear increase in Fermi arc length with temperature that

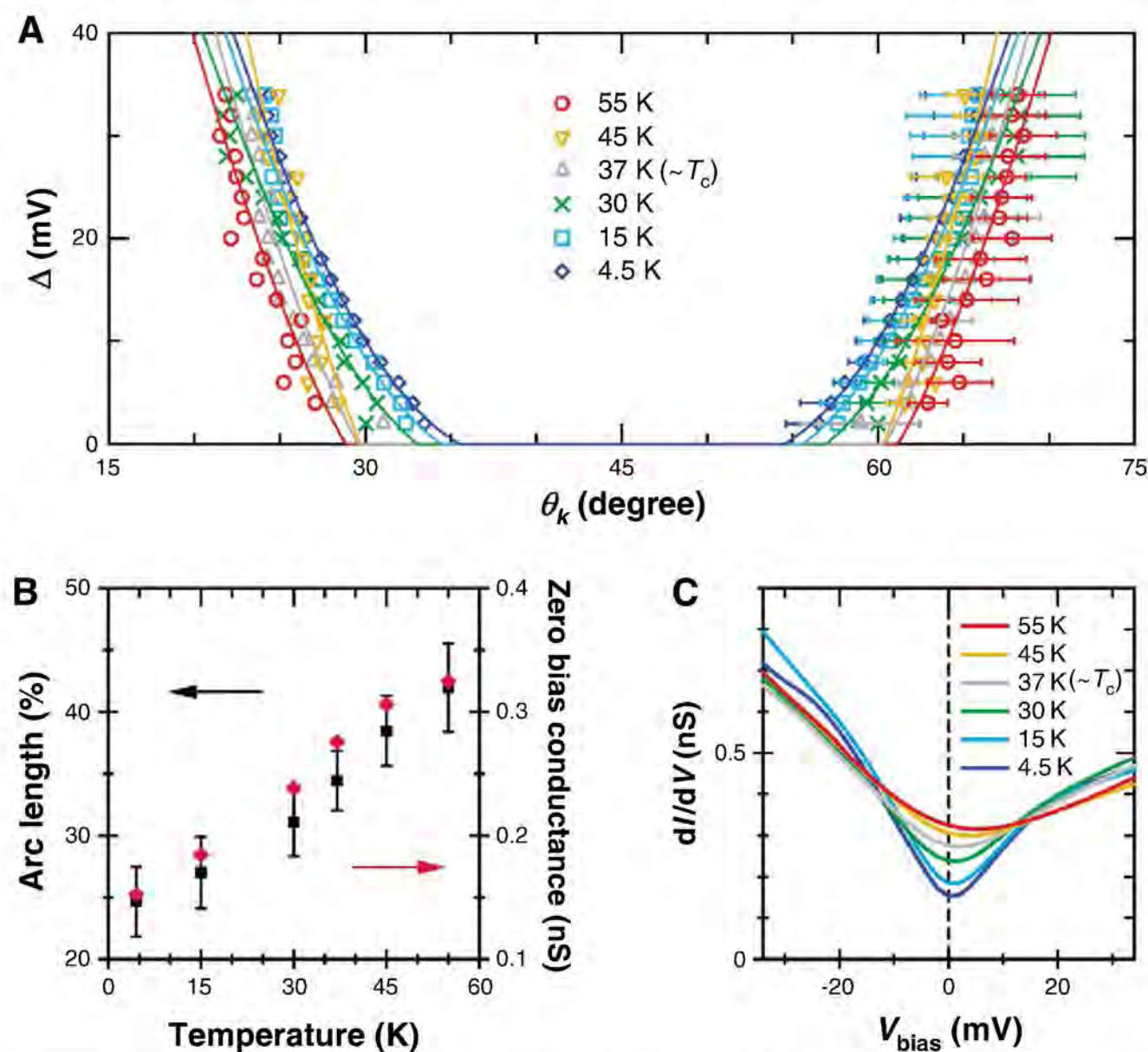


Fig. 3. (A) The superconducting energy gap $\pm|\Delta(\vec{k})|$ to coherent k -space Bogoliubov quasiparticle excitations [as determined from the internally consistent octet model analysis of $Z(\vec{q}, E)$] in the superconducting phase is shown for temperatures 4.5, 15, and 30 K. The energy gap magnitude $\pm|\Delta(\vec{k})|$ to coherent k -space excitations in the nonsuperconducting phase or pseudogap regime is shown for temperatures 37, 45, and 55 K. (B) The ungapped arc length from QPI analysis shows approximately linear temperature dependence. As previously reported in (22, 23, 30), there is an ungapped region even at the lowest temperatures in the superconducting state and, based on the observations shown here, this is probably indicative of combined effects of disorder and phase fluctuations from quantum mechanical sources. (C) The spatially averaged differential conductance is plotted as a function of temperature measured simultaneously with the data shown in Fig. 1. The high value of the zero-bias conductance is apparent even at low temperatures.

was originally observed in the pseudogap regime at higher doping (33).

We have emphasized that little distinguishes the observed octet QPI phenomenology between the superconducting phase and the underdoped pseudogap region (Figs. 1 to 3). However, Fig. 4 shows analysis of the energy and temperature dependence of the amplitudes of modulations due to scattering between k -space regions that have the same sign of the d-wave order parameter and between regions of opposite sign. Whereas the latter intensities drop precipitously as T rises (Fig. 4, B to D), the former pass right through T_c without appreciable changes (Fig. 4, F to H). This could be consistent with the d-wave Bogoliubov quasiparticle scattering processes, whose coherence factor products are insensitive to order parameter sign changes (30), remaining unperturbed by the loss of long-range phase rigidity in the pseudogap regime.

With the high $|\vec{q}|$ and E resolution and the enhanced sensitivity to very weak conductance variations introduced here, we were able to find the following characteristics for the low-energy quasiparticle interference modulations in the underdoped cuprate pseudogap regime: (i) The set of seven $\vec{q}_i(E)$ ($i = 1, 2, \dots, 7$) that are characteristic of the superconducting octet model are preserved unchanged upon passing above T_c (Fig. 1 and fig. S4). (ii) All the $\vec{q}_i(E)$ remain dispersive in a manner that is internally consistent with the octet phenomenology (Fig. 2 and fig. S4). (iii) The octet modulation wave vectors retain their particle-hole symmetry $\vec{q}_i(+E) = \vec{q}_i(-E)$. (iv) The modulations occur in the same energy range and emanate from the same contour in k -space (although at varying locations on that contour) as those observed at lowest temperatures (Fig. 3 and fig. S5). (v) The particle-hole-symmetric energy gap $\pm|\Delta(k)|$ moves away from the nodes with increasing T , leaving behind a growing arc of gapless excitations (Fig. 3). (vi) The intensities of modulations due to scattering between k -space regions having the same sign of a d-wave order parameter are maintained, whereas the intensities of those having a different sign are greatly diminished (Fig. 4).

These observations represent a number of advances in our understanding of the electronic structure of the cuprate pseudogap regime. First, because the basic QPI octet phenomenology is definitely the signature of d-wave superconductivity well below T_c (20–23), and because we show here that it evolves without pronounced changes through T_c , it seems implausible that it represents a different state above T_c . Moreover, the observed particle-hole-symmetric, dispersive, octet-model phenomenology is consistent with theoretical predictions for the QPI characteristics of a phase-incoherent d-wave superconductor (25–27). Our data therefore provide spectroscopic evidence confirming and extending the deductions reached in the transport and thermodynamic studies (13–17). In addition, knowledge of this spectroscopic signature may

now make it possible to explore the phase diagram regions exhibiting this state, to quantify the phase fluctuation rates with changing temperature (6) and doping, and to discriminate between different quantum fluctuation sources (7–12). Second, because all the $\vec{q}_i(E)$ observed in $Z(\vec{q}, E)$

disperse internally consistently with the octet model, they do not represent the signature of a static, ordered state in the underdoped pseudogap regime. Nevertheless, it is equally important to emphasize that the true nondispersive and symmetry-breaking excitations that occur at the

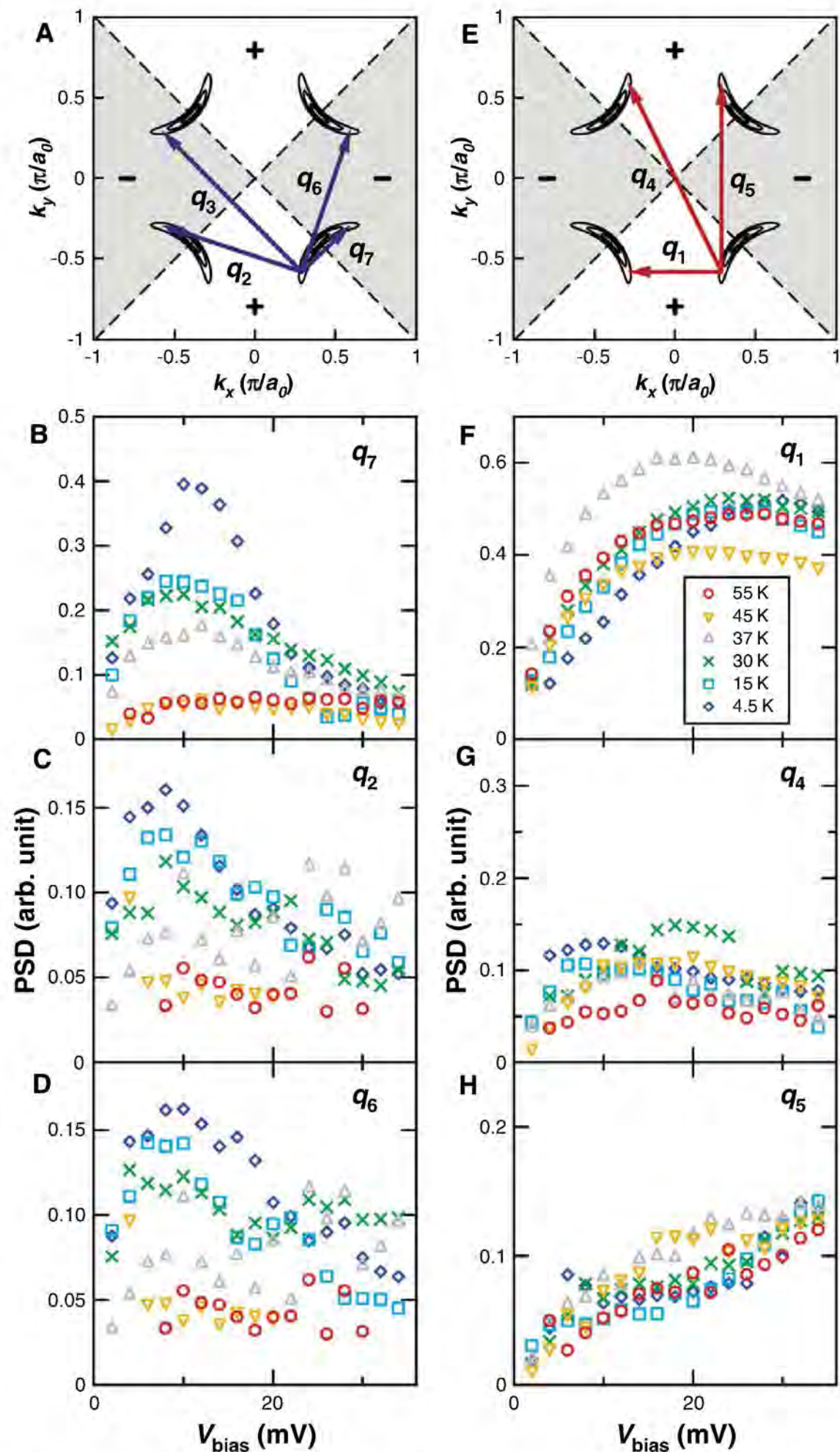


Fig. 4. (A) The octet q -vectors due to scattering between regions of k -space with different signs of a d-wave order parameter are indicated. (B to D) The power spectral densities for modulations with q_7 , q_2 , and q_6 at each energy and for different temperatures are shown. These modulations show a rapid decrease in intensity as temperature is increased. (E) The octet q -vectors due to scattering between regions of k -space with the same sign of a d-wave order parameter are indicated. (F to H) The power spectral densities for modulations q_1 , q_4 , and q_5 at each energy and for different temperatures are plotted. The intensity of these modulations shows no trend as temperature is increased and no particular indication of where the transport T_c occurs.

pseudogap energy scale (23, 29) coexist with these low-energy dispersive modulations at all temperatures studied. Third, analysis of the data using the octet model provides a new perspective on the superconducting energy gap at lowest dopings, indicating that an arc of gapless excitations exists in the strongly underdoped superconducting phase (22, 23, 30) and that it expands linearly in temperature. Such an arc might exist either due to effects of scattering (31, 32) or due to phase fluctuations (6) that are generated by a purely quantum mechanical processes (7–12, 34), or both. Fourth, as the alterations in interference intensities (Fig. 4) that are detected with increasing temperature mimic those generated by introduction of static vortices at low temperatures (30), it may be that they occur here for similar reasons but now in a nonstatic vortex fluid (13–17). Finally, our observations reveal that the electronic structure of the strongly underdoped pseudogap regime contains not two, but three fundamental components: (i) the Fermi arc (24), (ii) the gap $\pm|\Delta(\vec{k})|$ of a phase-disordered superconductor to coherent k -space excitations, and (iii) the nondispersive and locally symmetry-breaking excitations at the pseudogap energy scale (23, 29).

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S8

References

Movies S1 to S6

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Strong Coupling Between Single-Electron Tunneling and Nanomechanical Motion

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Nanoscale resonators that oscillate at high frequencies are useful in many measurement applications. We studied a high-quality mechanical resonator made from a suspended carbon nanotube driven into motion by applying a periodic radio frequency potential using a nearby antenna. Single-electron charge fluctuations created periodic modulations of the mechanical resonance frequency. A quality factor exceeding 10^5 allows the detection of a shift in resonance frequency caused by the addition of a single-electron charge on the nanotube. Additional evidence for the strong coupling of mechanical motion and electron tunneling is provided by an energy transfer to the electrons causing mechanical damping and unusual nonlinear behavior. We also discovered that a direct current through the nanotube spontaneously drives the mechanical resonator, exerting a force that is coherent with the high-frequency resonant mechanical motion.

Nanomechanical systems (1, 2) have promising applications, such as ultrasensitive mass detection (3–5). The combination of a high resonance frequency and a small mass also makes nanomechanical resonators attractive for a

fundamental study of mechanical motion in the quantum limit (6–9). For a successful observation of quantum motion of a macroscopic object, a high-frequency nanoscale resonator must have low dissipation (which implies a high quality factor Q) and a sensitive detector with minimum back-action (i.e., quantum limited) (10, 11). In our experiment, we demonstrate a dramatic back-action that strongly couples a quantum-dot detector to the resonator dynamics of a carbon nanotube, and which, in the limit of strong feedback, spon-

taneously excites large-amplitude resonant mechanical motion.

Nanomechanical resonators have been created by etching down larger structures. In small devices, however, surface effects impose an upper limit on Q (2). Alternatively, suspended carbon nanotubes can be used to avoid surface damage from the (etching) fabrication process. We recently developed a mechanical resonator based on an ultraclean carbon nanotube with high resonance frequencies of several hundred megahertz and a Q exceeding 10^5 (12). Here, we use this resonator to explore a strong coupling regime between single-electron tunneling and nanomechanical motion. We followed the pioneering approaches in which aluminum single-electron transistors were used as position detectors (6–8) and atomic force microscopy cantilevers as resonators (13–15); however, our experiment is in the limit of much stronger electro-mechanical coupling, achieved by embedding a quantum-dot detector in the nanomechanical resonator itself.

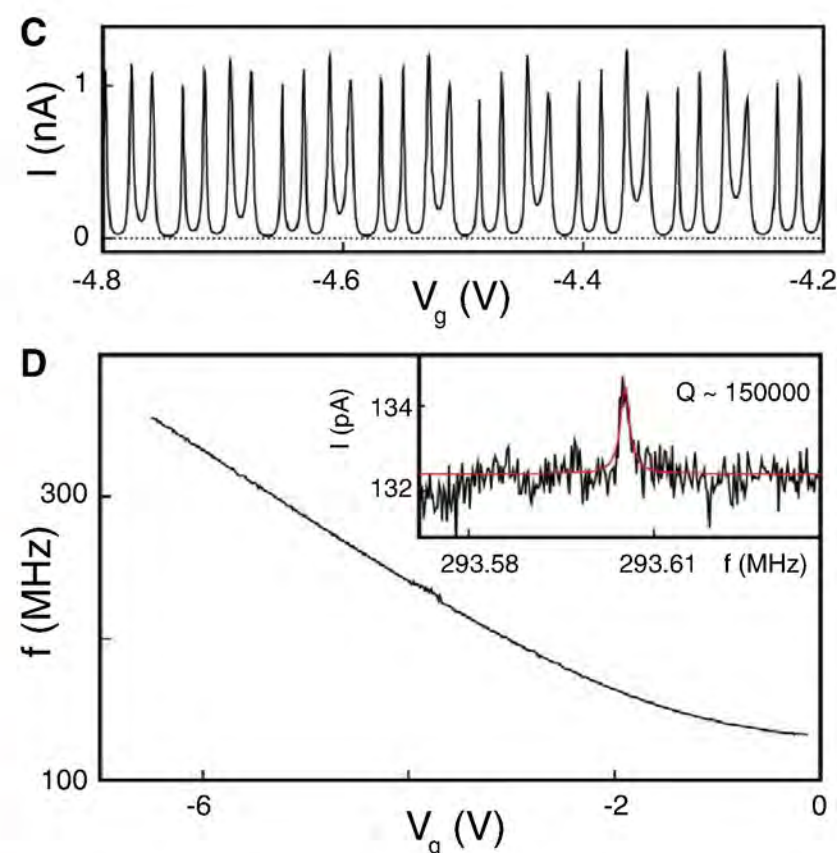
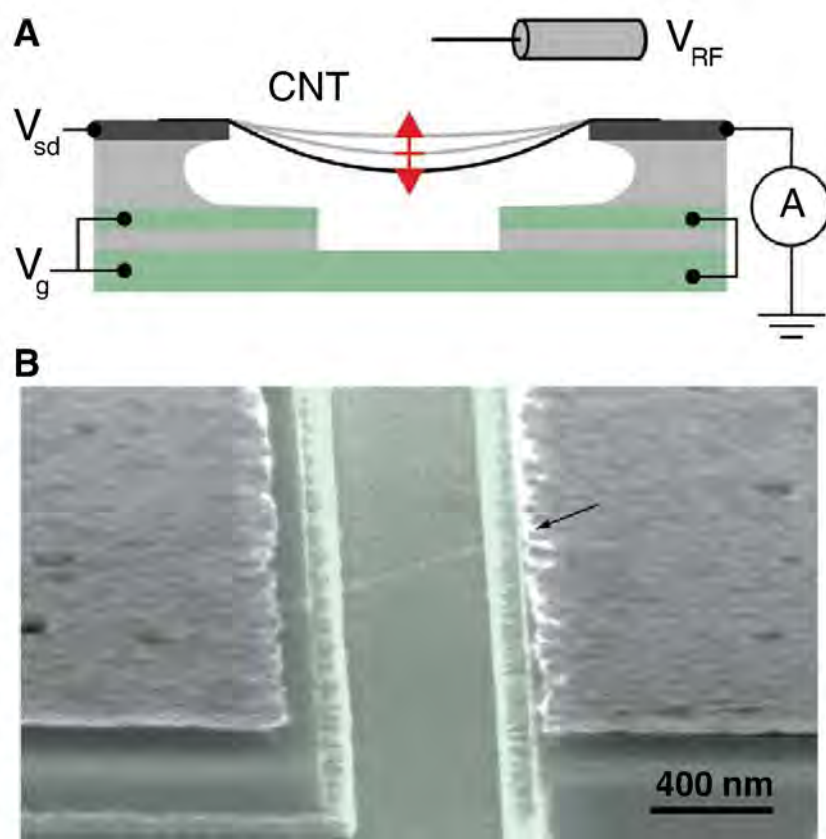
Our device consists of a nanotube suspended across a trench that makes electrical contact to two metal electrodes (Fig. 1). Electrons are confined in the nanotube by Schottky barriers at the Pt metal contacts, forming a quantum dot in the suspended segment. The nanotube growth is the last step in the fabrication process, yielding ultraclean devices (16), as demonstrated by the fourfold shell filling of the Coulomb peaks (Fig. 1C). We performed all measurements at 20 mK with an electron temperature of ~ 80 mK.

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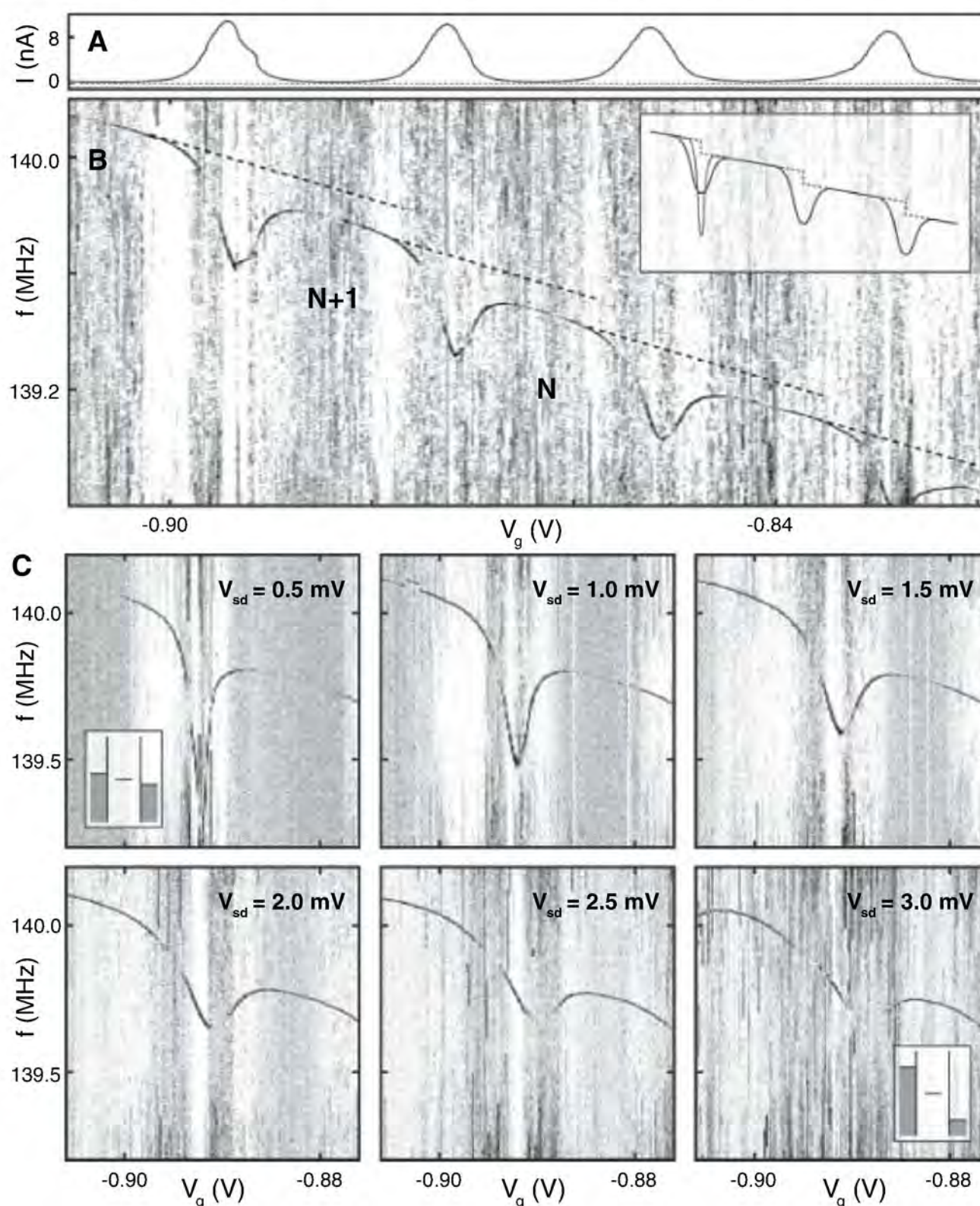
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Fig. 1. A high- Q nanotube mechanical resonator with an embedded quantum dot. **(A)** Device layout. A suspended carbon nanotube is excited into mechanical motion by applying an ac voltage to a nearby antenna. A dc current through the nanotube detects the motion. V_{RF} , radio frequency voltage; CNT, carbon nanotube. **(B)** Scanning electron microscopy image of a typical device. The arrow indicates the position of a nanotube. **(C)** A quantum dot, formed between Schottky barriers at the metal contacts, displays fourfold shell filling of holes.



(D) (Inset) The mechanical resonance induces a corresponding resonance in the dc current which can have a narrow linewidth with Q up to 150,000. (Main plot) The resonance frequency can be tuned using a tensioning force from the dc voltage on the gate.

Fig. 2. Single-electron tuning. **(A)** Nanotube current versus gate voltage showing single-electron tunneling at the peaks and Coulomb blockade in the valleys. This curve is taken from **(B)** at $f = 138.8$ MHz. **(B)** Normalized resonance signal $\Delta/\Delta_{\text{peak}}$ (see SOM) versus RF frequency and gate voltage ($V_{sd} = 1.5$ mV). The tuned mechanical resonance shows up as the darker curve with dips at the Coulomb peaks. The offsets between the dashed lines indicate the frequency shift due to the addition of one electron to the nanotube. The resonance frequency also shows dips caused by a softening of the spring constant because of single-electron charge fluctuations. N , number of holes on the quantum dot. (Inset) The expected resonance behavior (see text). **(C)** Zoom-in view on one frequency dip for various source-drain voltages (V_{sd}) showing dip broadening for increasing V_{sd} . (Insets) Energy diagrams for small and large V_{sd} .



We actuate the resonator with a nearby antenna and detect the resonator motion by its influence on the dc current through the nanotube. The inset to Fig. 1D shows a peak in the current at the resonance frequency, which we have identified as a bending-mode mechanical resonance of the nanotube (12). Q typically exceeds 10^5 , which is an increase of more than two orders of magnitude compared with previous nanotube studies (4, 17, 18). The resonance frequency is tuned by more than a factor of 2 with the gate voltage (Fig. 1D). The electric field from the gate pulls the nanotube toward it, and the subsequent lengthening of the nanotube induces more tension, similar to the tuning of a guitar string (17).

Our detection signal results from a change in the gate capacitance (ΔC_g) during a displacement of the nanotube. This changes the effective quantum-dot potential and, if positioned initially beside a Coulomb peak (Fig. 1C), can move it onto the peak, thereby increasing the current. For a nanotube oscillating on resonance, the effective potential oscillates, and the nonlinearity of Coulomb blockade allows it to be rectified to a detectable dc current.

The narrow linewidth of the resonance peak provides an unprecedented sensitive probe for studying nanomechanical motion. We first show

the influence of a single electron on the resonance frequency (f_0). The Coulomb oscillations in Fig. 2A are caused by single-electron tunneling giving rise to current peaks, and Coulomb blockade fixes the electron number in the valleys. From valley to valley, the electron number changes by one. Figure 2B shows the mechanical resonance signal recorded at the same time. Overall, a more negative gate voltage (right to left) increases the total charge on the nanotube, increasing the tension. This process stiffens the mechanical spring constant and increases the resonance frequency. Linear stiffening occurs in the Coulomb valleys (indicated with dashed lines), whereas at Coulomb peaks, a peculiar softening occurs, which is visible as dips in f_0 .

We first focus on the change in resonance frequency caused by the addition of one electron, which is measured as offsets of ~ 0.1 MHz between the dashed lines. This shift from single-electron tuning, predicted in (19), is ~ 20 times our linewidth and thus clearly resolvable. Because we compare valleys with a fixed electron number, this single-electron tuning comes from a change in a static force on the nanotube. The (electro-) static force is proportional to the square of the charge on the nanotube and, thus, adding one electron charge results here in a detectable shift in the mechanical resonance (19). The shifts from

single-electron tuning can be as large as 0.5 MHz, more than 100 times the line width (20).

Next we focus on the dips in resonance frequency that occur at the Coulomb peaks. The current at the Coulomb peaks is carried by single-electron tunneling, meaning that one electron tunnels off the nanotube before the next electron can enter the tube. The charge on the nanotube fluctuates by exactly one electron charge (e) with a time dynamics that can be accounted for in detail by the theory of Coulomb blockade (21). The average rate (Γ) at which an electron moves across the tube can be read off from the current $I = e\Gamma$ (1.6 pA corresponds to a 10-MHz rate). Moving the gate voltage off or on a Coulomb peak, we can tune the rate from the regime $\Gamma \sim f_0$ to $\Gamma \gg f_0$ and explore the different effects on the mechanical resonance.

In Fig. 2, A and B, the Coulomb peak values of ~ 8 nA yield $\Gamma \sim 300f_0$, the regime of many single-electron tunneling events per mechanical oscillation. In addition to the static force and the radio frequency (RF) oscillating driving force, single-electron tunneling now exerts a time-fluctuating, dynamic force on the mechanical resonator. We observe that this dynamic force causes softening, giving dips in the resonance frequency. The single-electron charge fluctua-

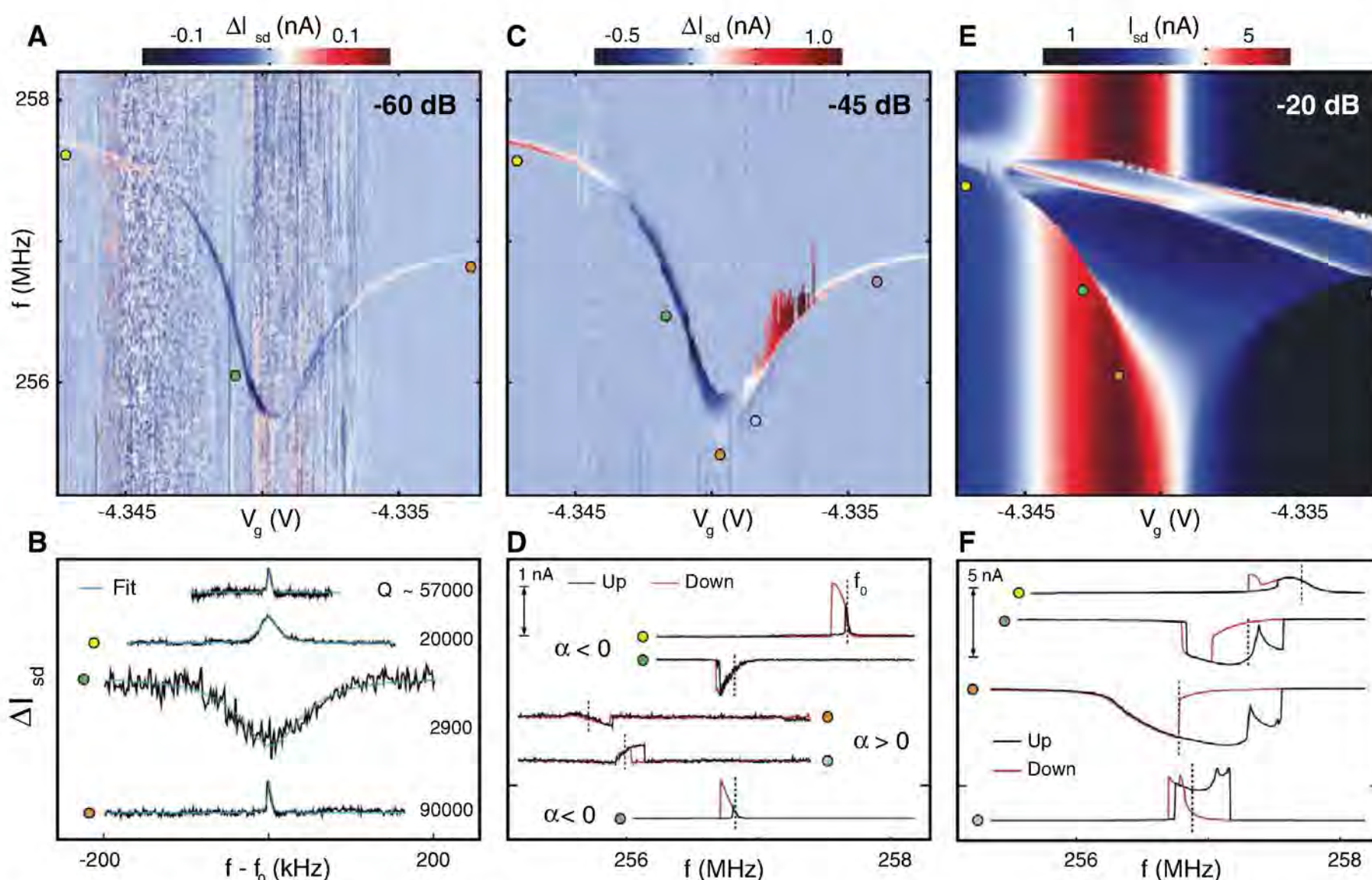


Fig. 3. Line shapes of the mechanical resonance from linear to nonlinear driving regimes. **(A)** Detector current (ΔI) versus frequency and gate-voltage at RF excitation power of -60 dB, as the gate voltage is swept through one Coulomb peak. **(B)** Fits of the resonance to a squared Lorentzian line shape at different gate voltages (12). The RF power for each trace is adjusted to stay in the linear driving regime (-75 , -64 , -52 , and -77 dB, top to bottom). Traces are taken at the positions indicated by colored circles (aside from the top trace,

which is taken at $V_g = -4.35$ V). **(C)** At -45 dB, the resonance has an asymmetric line shape with one sharp edge; see linecuts **(D)**, typical for a nonlinear oscillator (23, 24). Dashed lines in **(D)** and **(F)** indicate resonance frequency f_0 at low powers. **(E and F)** At even higher driving powers (-20 dB), the mechanical resonator displays sharp subpeaks and several jumps in amplitude when switching between different stable modes. **(C)** and **(E)** are taken in the upward sweep direction (downward sweeps shown in the SOM).

tions do not simply smooth the stepwise transition from the static single-electron tuning shifts. Instead, fluctuations caused dips in the resonant frequency up to one order of magnitude greater than the single-electron tuning shifts. As shown in (13, 22) and discussed in detail in the supporting online material (SOM) (20), the dynamic force modifies the nanotube's spring constant (k) resulting in a softening of the mechanical resonance. The shape of the frequency dip can be altered by applying a finite bias (V_{sd}) across the nanotube. Starting from deep and narrow at small $V_{sd} = 0.5$ mV, the dip becomes shallower and broader with increasing V_{sd} . This dip shape largely resembles the broadening of Coulomb blockade peaks that occurs with increasing V_{sd} . Thus, we conclude from Fig. 2 that the single-electron tuning oscillations are a mechanical effect that is a direct consequence of single-electron tunneling oscillations.

Besides softening, the charge fluctuations also provide a channel for dissipation of mechanical energy. Figure 3A shows the resonance dip for small RF power, with frequency traces in Fig. 3B. In the Coulomb valleys, tunneling is suppressed

($\Gamma \sim f_0$), damping of the mechanical motion is minimized, and we observe the highest value of Q . On a Coulomb peak, charge fluctuations are maximized ($\Gamma \gg f_0$), and Q decreases to a few thousand. These results explicitly show that detector back-action can cause substantial mechanical damping. The underlying mechanism for the damping is an energy transfer occasionally occurring when a current-carrying electron is pushed up to a higher (electrostatic) energy by the nanotube motion before tunneling out of the dot. This gain in potential energy is later dissipated in the drain contact.

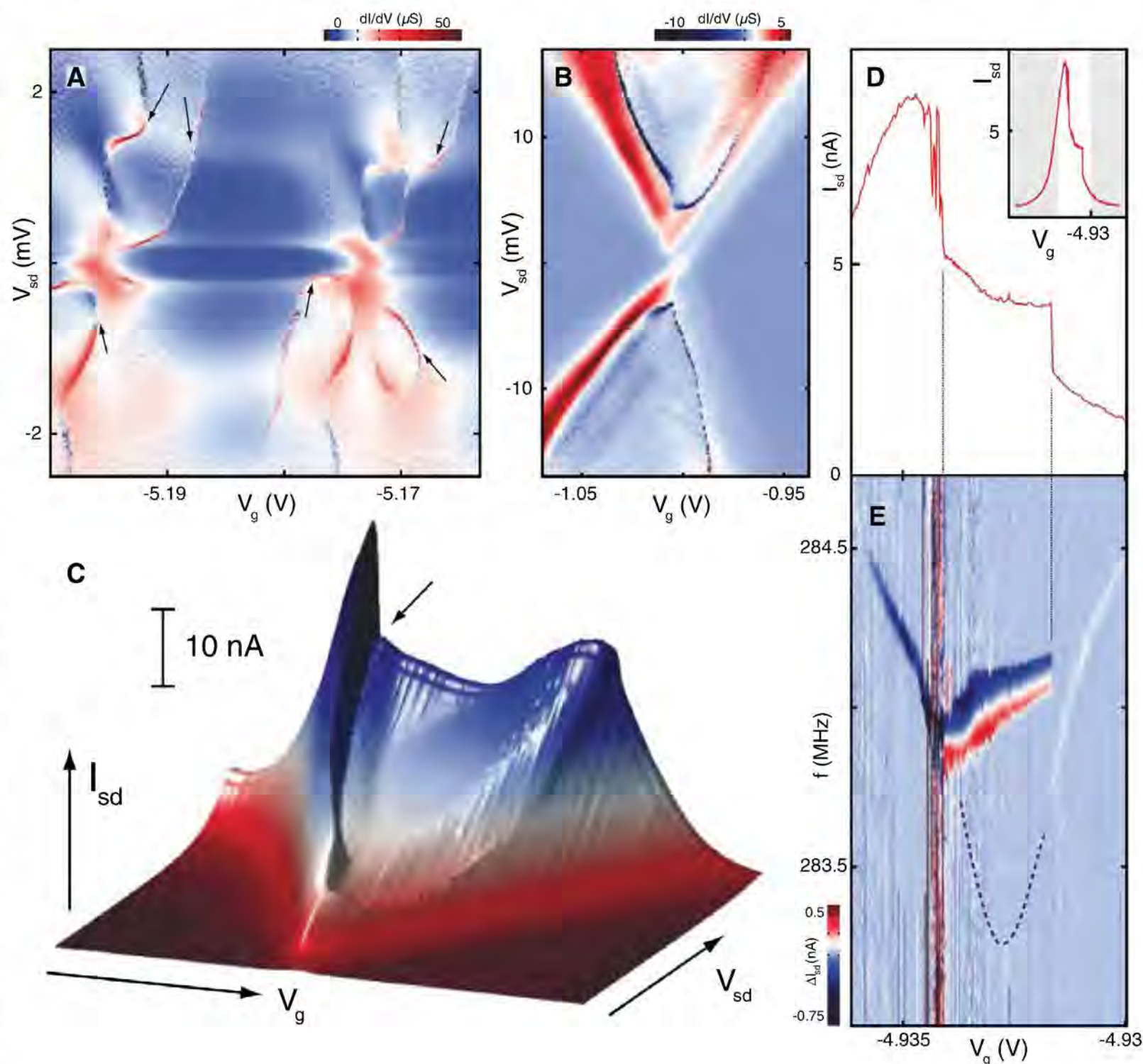
If we drive the system at higher RF powers (Fig. 3, C and D), we observe an asymmetric resonance peak, along with distinct hysteresis between upward and downward frequency sweeps. Theoretically, this marks the onset of nonlinear terms in the equation of motion, such as in the well-studied Duffing oscillator (23, 24). k is modified by a large oscillation amplitude (x) that is accounted for by replacing k with $(k + \alpha x^2)$. The time-averaged spring constant increases if $\alpha > 0$, which is accompanied by a sharp edge at the high-frequency side of the peak; vice versa for $\alpha < 0$. In

addition to the overall softening of k yielding the frequency dips of Fig. 2, the fluctuating charge on the dot also changes α , giving a softening spring ($\alpha < 0$) outside of the frequency dip (Coulomb valleys) and a hardening spring ($\alpha > 0$) inside the frequency dip (Coulomb peaks), as shown in Fig. 3. The sign of α follows the curvature of $f_0(V_g)$ induced by the fluctuating electron force, giving a change in sign at the inflection point of the frequency dip. Nonlinearity from the single-electron force in our device dominates and is much stronger than that from the mechanical deformation (20).

Figure 3, E and F, shows the regime of further enhanced RF driving. Now the nonlinearity is no longer a perturbation of the spring constant, but instead gives sharp peaks in the line shape and switching between several different metastable modes [see further data in the SOM (20)]. At this strong driving, we observe highly structured nonlinear mechanical behavior that arises from the coupling of the resonator motion to the quantum dot.

In Fig. 3, we studied nonlinear coupling between the quantum dot and the mechanical resonator by applying a large RF driving force at a

Fig. 4. Spontaneous driving of the mechanical resonance by single-electron tunneling. (A) Differential conductance (dI/dV_{sd}) showing ridges of sharp positive (deep red) and negative (deep blue) spikes (arrows). (B) Similar ridges measured on device two (trench width = 430 nm) in the few-hole regime (four-hole-to-three-hole transition). Spikes in dI/dV_{sd} appear as steplike ridges in current. (C) shows the data from the upper half of (B), but now as a three-dimensional current plot. The ridges are entirely reproducible. (D) (Inset) Coulomb peak at $V_{sd} = 0.5$ mV showing large switching-steps. (Main plot) Zoom-in view on data from inset. (E) RF driven mechanical resonance measured for the same Coulomb peak in (D) at a driving power of -50 dB. Outside the switch region, the resonance has a narrow line shape and follows the softening dip from Figs. 2 and 3. At the first switch, the resonance position departs from the expected position (indicated by dashed line). The mechanical signal is strongly enhanced in amplitude and displays a broad asymmetric line shape. At the second switch, the resonance returns to the frequency and narrow line shape expected at these powers.



small V_{sd} . In Fig. 4, we consider small or absent RF driving force and now apply a large V_{sd} across the quantum dot. Figure 4A shows a standard Coulomb blockade measurement of the quantum dot. Mechanical effects in Coulomb diamonds have been studied before in the form of phonon sidebands of electronic transitions (25–28). Shown in the data of Fig. 4 are reproducible ridges of positive and negative spikes in the differential conductance as indicated by arrows. This instability has been seen in all 12 measured devices with clean suspended nanotubes and never in nonsuspended devices. Figure 4, B and C, shows such ridges in a second device, visible as both spikes in the differential conductance (Fig. 4B) and discrete jumps in the current (Fig. 4C). The barriers in device two were highly tunable: We found that the switch-ridge could be suppressed by reducing the tunnel coupling to the source-drain leads, thereby decreasing the current. The instability disappears roughly when the tunnel rate is decreased below the mechanical resonance frequency (see SOM) (20).

In a model predicting such instabilities (29), positive feedback from single-electron tunneling excites the mechanical resonator into a large-amplitude oscillation. The theory predicts a characteristic shape of the switch-ridges and the suppression of the ridges for $\Gamma \sim f_0$, in marked agreement with our observations. Such feedback also requires a very high Q , which may explain why it has not been observed in previous suspended quantum-dot devices (26, 28). If the required positive feedback is present, however, it should also have a mechanical signature; such a signature is demonstrated in Fig. 4E. The RF-

driven mechanical resonance experiences a dramatic perturbation triggered by the switch-ridge discontinuities in the Coulomb peak current shown in Fig. 4D. At the position of the switch, the resonance peak shows a sudden departure from the expected frequency dip (dashed line) and becomes strongly asymmetric and broad, as if driven by a much higher RF power. This is indeed the case, but the driving power is now provided by an internal source: Because of the strong feedback, the random fluctuating force from single-electron tunneling becomes a driving force coherent with the mechanical oscillation. Remarkably, the dc current through the quantum dot can be used both to detect the high-frequency resonance and, in the case of strong feedback, directly excite resonant mechanical motion.

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Figs. S1 to S6

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Coupling Mechanics to Charge Transport in Carbon Nanotube Mechanical Resonators

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Nanoelectromechanical resonators have potential applications in sensing, cooling, and mechanical signal processing. An important parameter in these systems is the strength of coupling the resonator motion to charge transport through the device. We investigated the mechanical oscillations of a suspended single-walled carbon nanotube that also acts as a single-electron transistor. The coupling of the mechanical and the charge degrees of freedom is strikingly strong as well as widely tunable (the associated damping rate is $\sim 3 \times 10^6$ Hz). In particular, the coupling is strong enough to drive the oscillations in the nonlinear regime.

Carbon nanotubes have been used to fabricate mechanical resonators that can be operated at ultrahigh frequencies, have widely tunable resonance frequencies, and can be used as ultrasensitive inertial mass sensors (1–10). In addition, carbon nanotubes also have exceptional electron transport properties, including ballistic conduction over long distances or multiple Coulomb blockade-related phenomena

that can be observed even up to room temperature (11). Coupling the mechanical motion of nanotube resonators to electron transport is thus highly appealing. In particular, we would like to use such a coupling as a way to control the mechanical motion at the nanoscale. Recently, micro-fabricated silicon resonators have been fabricated whose mechanical vibrations are damped by cooling from a nearby superconducting single-electron

transistor (12). In the case of nanotube resonators, however, the extent to which it is possible to couple mechanical vibrations and charge transport is not clear.

We studied the coupling between the mechanics and the electron transport of a single-walled carbon nanotube (SWNT) resonator at cryogenic temperature in which conducting electrons enter the Coulomb-blockade regime. We show that single electrons tunneling into and out of the nanotube greatly affect the nanotube motion. The coupling can be made stronger than in nanoelectromechanical systems (NEMS) resonators studied so far, including those fabricated in silicon by using top-down approaches. Moreover,

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the coupling allows us to modify the motion of the SWNT in an unprecedented manner. The resonance frequency, the quality factor, and the non-linear dynamic can be tuned by using an external electric means (voltage applied on a gate), which is convenient for practical use.

We fabricated devices using standard nanofabrication techniques (Fig. 1A). SWNTs were grown by means of chemical-vapor deposition on a highly doped Si substrate coated with a 1- μm -thick SiO_2 layer. SWNTs were connected to two Cr/Au electrodes (5nm/100nm) by use of electron-beam lithography. We performed wet etching in buffered hydrofluoric acid so as to suspend the nanotube. An annealing treatment at 400 C during 1 hour in a flow of Ar and H_2 was carried out in order to remove impurities (polymethyl methacrylate residues and other contaminants) from the SWNTs.

The SWNT motion was driven by applying an oscillating voltage $V_g^{\text{AC}} \cos(2\pi f t)$ on the gate (Fig. 1A), which generates an electrostatic force $F_{\text{drive}} \propto V_g^{\text{AC}} \cos(2\pi f t)$ on the SWNT. The induced motion was detected via a mixing technique (1). For this, we applied a second voltage on the source electrode $V_{\text{SD}}^{\text{AC}} \cos[2\pi(f + \delta f)t]$. The SWNT acted as a frequency mixer, and we measured the mixing current I_{mix} at the frequency component δf from the drain electrode (Fig. 1A). The line shape of I_{mix} as a function of f (Fig. 1B) allows us to extract the resonance frequency f_0 and the quality factor Q (13).

Figure 2A shows the electron transport properties of the device; the SWNT differential conductance G at 4 K is plotted as a function of the constant voltage V_g^{DC} applied on the gate and the constant voltage $V_{\text{SD}}^{\text{DC}}$ applied on the source. G oscillated with V_g^{DC} in a way that is typical of the Coulomb-blockade regime. As for the mechanical properties, Fig. 2B shows that some features of I_{mix} oscillate with V_g^{DC} with the same period as for G (such as the shape of the red lobes). The correlation between the electrical conductance and the mechanical motion becomes clearer in Fig. 3, A, C, and E, where we plot G at zero-bias, f_0 , and Q as functions of V_g^{DC} . Indeed, G is the maximum, whereas f_0 and Q are low. This correlation suggests that the mechanical motion is influenced by Coulomb blockade.

Charge transport through SWNTs in the Coulomb-blockade regime is a well-studied phenomenon. When sweeping V_g^{DC} , the conductance oscillates, whereas the charge q_{dot} residing on the nanotube dot increases stepwise (Fig. 1, C and D). In our experiment, the nanotube is also behaving as a mechanical resonator (with resonance frequency f_0 , effective spring constant k , and effective mass m). The capacitance C_g between the SWNT and the gate oscillates as $\delta C_g = C_g' \delta z$ (where δz is the SWNT deflection and C_g' the derivative of C_g with respect to δz). As a result, the SWNT is repeatedly charging and discharging by the amount δq_{dot} .

This charge oscillation causes damping of the mechanical motion because δq_{dot} has to flow through the tunnel resistances at the SWNT-electrode interfaces (Fig. 3B) and the energy

dissipated by the current is supplied from the mechanical resonator. This dissipation is equivalent to an out-of-phase force acting on the SWNT (13–15). Consequently, the oscillating motion is delayed (by a phase $\propto 1/Q$) as compared with when no electrons are tunnelling onto the SWNT.

The oscillating charge on the SWNT also results in a shift of the resonance frequency of the resonator. The reason is that the flow of the charge modifies the electrostatic force $F_e = \frac{1}{2} C_g' (V_g^{\text{DC}} - V_{\text{dot}})^2$ between the gate and the nanotube (V_{dot} is the nanotube potential and depends on δq_{dot}). Indeed, it can be shown that F_e oscillates as a spring force (13). This modifies the spring constant of the SWNT resonator by δk . The resonator softens or hardens, and the resonance frequency changes by the amount $\delta f_0 = f_0/2 \cdot \delta k/k$.

This damping and the resonance frequency are expected to oscillate with V_g^{DC} because the amplitude of the δq_{dot} oscillation depends on V_g^{DC} (the amplitude is large when q_{dot} increases sharply with V_g^{DC} and low when q_{dot} increases

weakly with V_g^{DC}) (Fig. 1C, green segment). Q and δf_0 can be expressed as (13)

$$1/Q = 2\pi f_0 \frac{C_g'^2}{k} (V_g^{\text{DC}})^2 \left(\frac{2}{\Gamma C_{\text{dot}}} \right)^2 G \quad (1)$$

$$\delta f_0 = -\frac{f_0}{2} \frac{C_g'^2}{k} \frac{(V_g^{\text{DC}})^2}{C_{\text{dot}}} \left(\frac{2G}{C_{\text{dot}}\Gamma} - 1 \right) \quad (2)$$

where Γ is the tunnelling rate through the nanotube-electrode interface and C_{dot} is the total dot capacitance. Equation 1 and 2 are valid for $f_0 \ll \Gamma$ and in the so-called quantum regime of Coulomb blockade, in which the thermal energy $k_B T$ (where k_B is Boltzmann's constant and T is the temperature) is lower than the level spacing (the energy separation due to quantum confinement). Equations 1 and 2 show that Q and f_0 oscillate as a function of V_g^{DC} with the same period as for G , which is in agreement with the experiments. We compared the measurements to Eqs. 1 and 2 using the measured G in Fig. 3A and taking

Fig. 1. (A) Schematic of the mechanical resonator. The moveable part is a SWNT (oscillations indicated by a red arrow). The SWNT length is 1 μm and the diameter is ~ 1.1 nm. The motion is capacitively driven by the application of an oscillating voltage on the gate. The nanotube position is detected by applying an oscillating voltage on the source electrode and measuring the current from the drain electrode. $\delta f = 10$ kHz. (B) Measured mechanical resonance (mixing current as a function of driving frequency). The red curve is a fit of the resonance. (C and D) Schematic of the charge on the SWNT and its conductance as a function of the control charge ($q_c = -C_g V_g^{\text{DC}}$) in the Coulomb-blockade regime. Vibrations cause the charge on the SWNT to oscillate (δq_{dot} and Δq_{dot}).

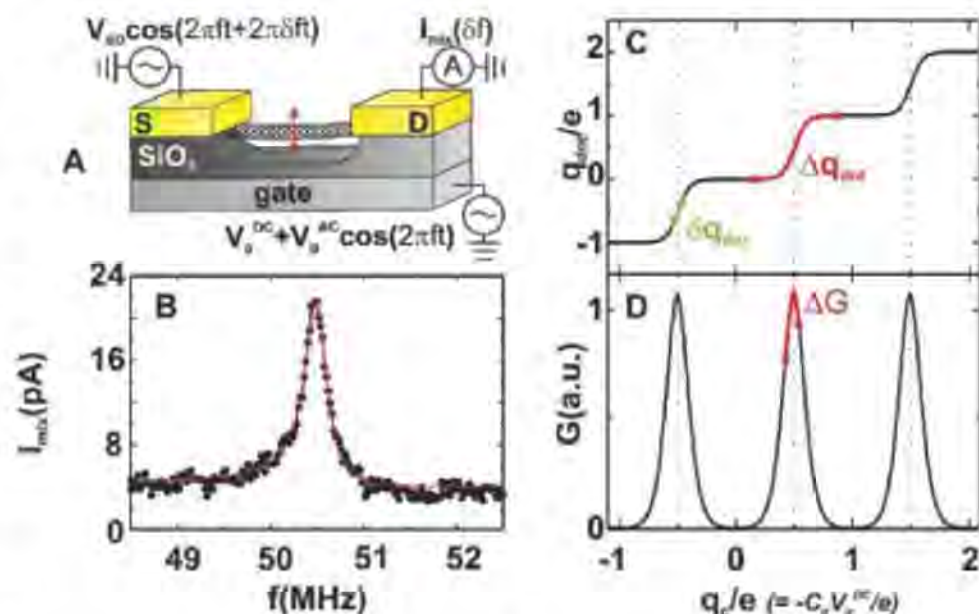
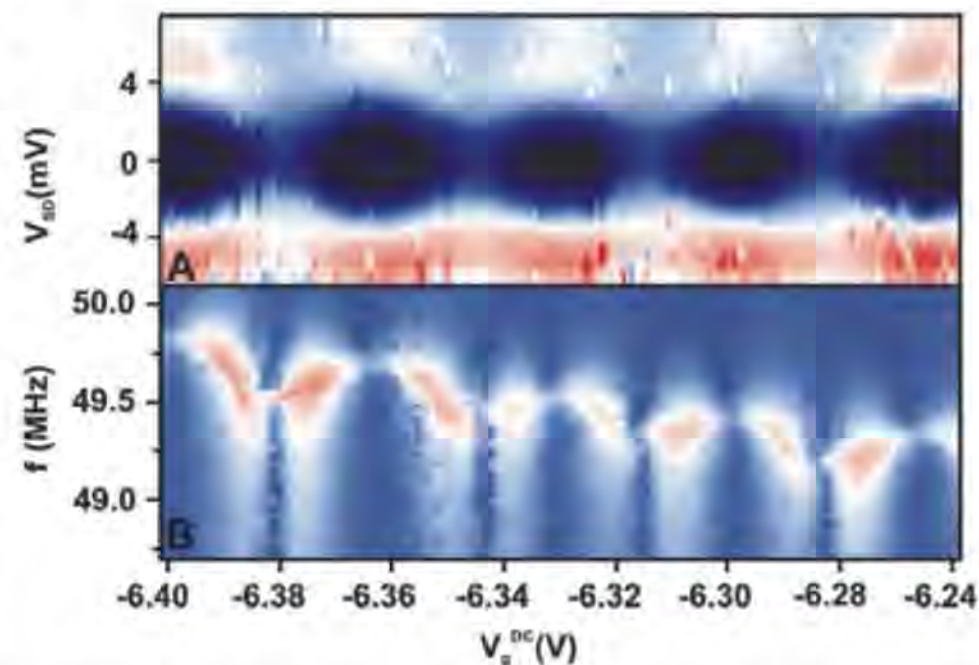


Fig. 2. Electronic and mechanical properties of the device at 4 K. (A) The SWNT differential conductance as a function of source-drain voltage and gate voltage. The measurements are consistent with Coulomb blockade. Blue corresponds to low conductance, and red corresponds to high conductance. (B) Mixing current from the drain as a function of driving frequency and gate voltage. The frequency of the red lobes (related to the mechanical resonance) goes up and down when increasing V_g^{DC} with the same period as for the SWNT conductance. This shows that the mechanics and the charge transport in the device are correlated. In addition, the frequency of the red lobes shifts continuously over the full V_g^{DC} sweep caused by mechanical tension (the static electrostatic force bends the nanotube). V_g^{AC} is 0.5 mV and $V_{\text{SD}}^{\text{AC}}$ is 0.1 mV (f_0 and Q remain the same when decreasing V_g^{AC} to 0.15 mV at 4K). Blue corresponds to low current, and red corresponds to high current.



$C_g'^2/k$ and Γ as fitting parameters (16). Figure 3, D and F, shows that the agreement is rather good. The values obtained for $C_g'^2/k$ and Γ were in quite reasonable agreement with theory (17).

These results show that the coupling between mechanical oscillations and the charge transport is the main dissipation mechanism at low temperature in the SWNT resonator (18). Our work shows that an obvious fabrication strategy in the future in order to obtain higher Q at low tem-

perature is to reduce the resistance at the SWNT-electrode interfaces ($Q \propto \Gamma$). Other dissipation mechanisms such as thermoelastic damping dominate $1/Q$ at higher temperatures. Indeed, $1/Q$ associated to the coupling between the mechanics and the electron transport is expected to be about 10^{-7} (13), whereas the measured $1/Q$ is $1/40$.

We considered measurements in which the large driving force applied to the SWNT accesses the nonlinear dynamic of the motion. Figure 4, A

and B, shows two resonance peaks at 1.5 K (mixing current as a function of driving frequency). The resonance in Fig. 4A has a surprising shape; it splits in two peaks, a broad and a narrow one. The narrow peak appears only at gate voltages near the Coulomb blockade conductance peaks (Fig. 4B, inset, and Fig. 4C). The most striking feature is that its width becomes narrower when increasing the driving force. The (effective) quality factor can increase up to about 5100 (Fig. 4E).

The coupling between the mechanics and the charge transport plays a central role in this nonlinear dynamic, as illustrated in Fig. 1C and by considering large mechanical oscillation amplitude (large oscillations of C_g). During one oscillation cycle, the charging and discharging of the dot is highly nontrivial because of the stepwise dependence of q_{dot} on V_g^{DC} (Fig. 1C, red trace Δq_{dot}). As a result, F_e depends nonlinearly on δz in contrast to the linear dependence at low oscillation amplitude. We have carried out numerical simulations to quantify this force and its effect on the motion. Specifically, the nanotube displacement and the force F_e have been calculated iteratively as a function of time in a self-consistent manner. The oscillating voltages applied on the gate and the source at frequencies f and $f + \delta f$, respectively, have been included in order to obtain the current from the drain as a function of time. A limit cycle in the simulation is obtained. Full details of our simulations are provided in (13). The calculations predict the appearance of an extra peak at gate voltages close to the Coulomb-blockade conductance peaks as well as a V_g^{DC} dependence of the resonance frequency that are in agreement with the experiments (Fig. 4, C and D). In addition, the simulations show that the width of the extra peak becomes narrower when increasing V_g^{AC} , but the effect is somewhat smaller than observed in the experiment (Fig. 4, E and F). Overall, it is remarkable that the calculations can capture the main experimental findings, especially when considering the simplicity of the model. The model may be improved by the incorporation of additional effects, such as mechanical-related nonlinearities that are expected to be important in SWNTs (19).

The double-peak structure in the simulations arises from the interplay between two nonlinear phenomena: one associated with the nonlinear dynamic of the motion (discussed above) and the other with the nonlinear detection mechanism. The latter nonlinearity becomes relevant when the amplitude of the mechanical motion is sufficient to drive G through the Coulomb blockade peak (Fig. 1D, red trace ΔG). The simulations, in that case, show that the induced current is lowered. This reduction of the current occurs only for large δz so that a resonance in position (δz as a function of f) is detected in $I_{\text{mix}}(f)$ as a double peak structure. If the shape of the resonance in δz was a Lorentzian, the two peaks in I_{mix} would have the same shape. However, the resonance in position has a sharkfin shape [Duffing-like resonance (20)] because of the nonlinearity of q_{dot} on δz so that one peak in I_{mix} is narrower (Fig. 4F, inset). Because the second peak

Fig. 3. Electronic and mechanical properties of the device at 4 K. (A) Conductance of the SWNT as a function of gate voltage. (B) Schematic of the dissipation process. The mechanical oscillation charges and discharges the SWNT by the amount δq_{dot} , which results in a current δI_{dot} flowing through the resistance at the nanotube-electrode interface. (C and D) Measured and calculated resonance frequency as a function of gate voltage. (E and F) Measured and calculated quality factor as a function of gate voltage.

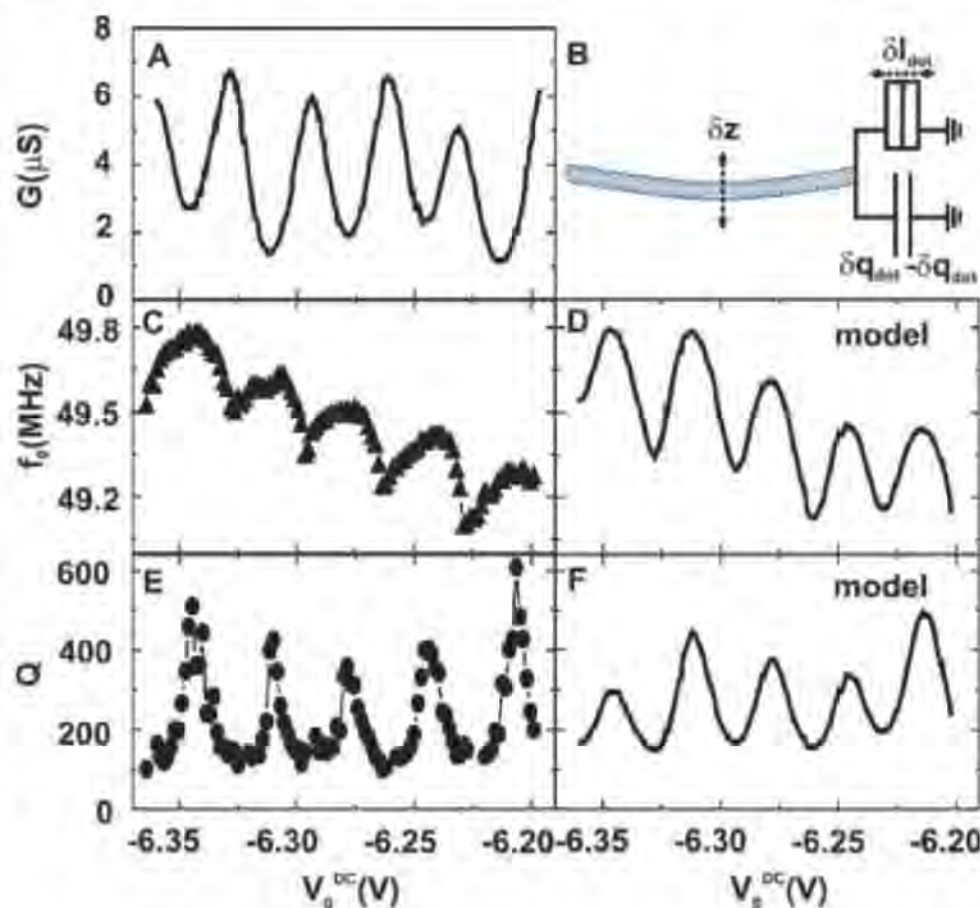
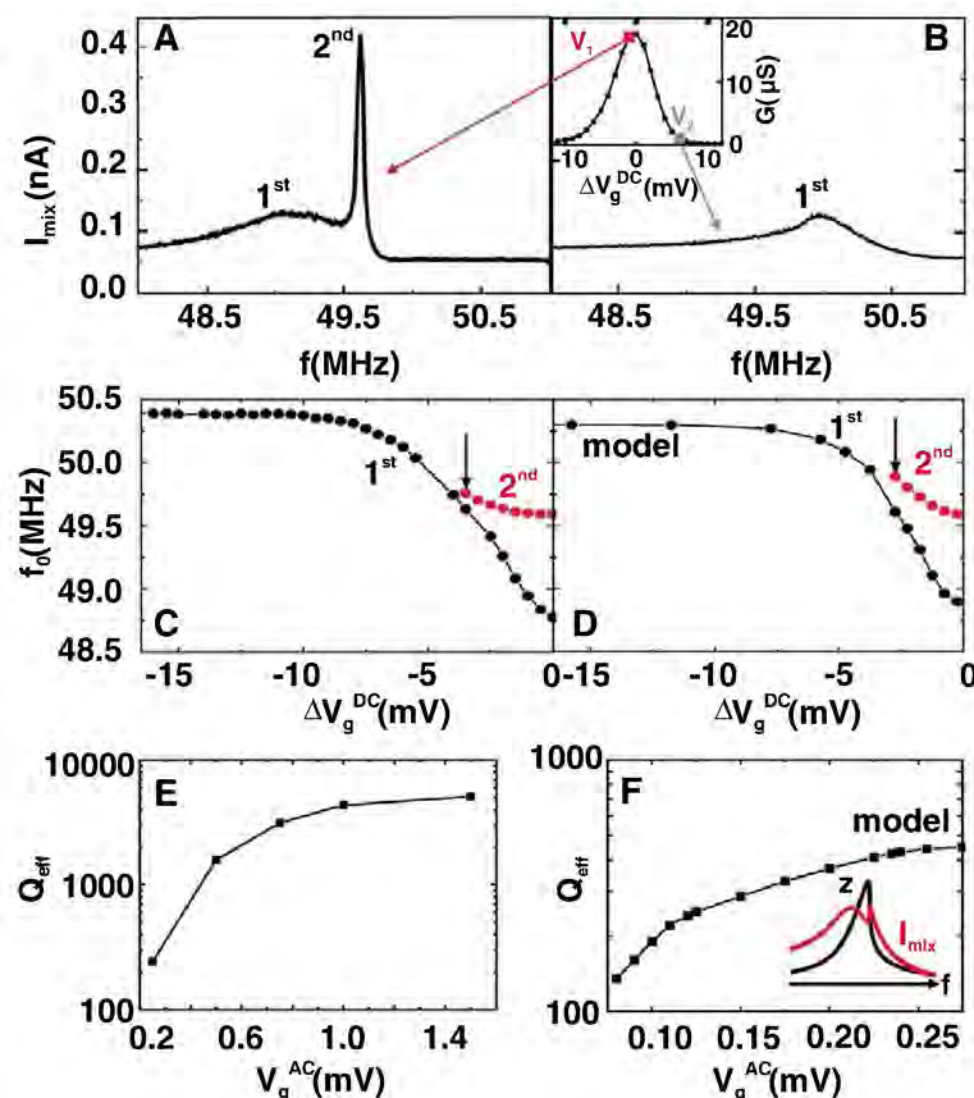


Fig. 4. Nonlinear dynamic of the SWNT motion at 1.5 K. (A and B) Mixing current as a function of driving frequency for two different gate voltages. A second peak appears in (A) that is very narrow. The inset shows the nanotube conductance as a function of gate voltage. ΔV_g^{DC} is measured from the maximum of the Coulomb-blockade peak. (C and D) Measured and calculated resonance frequency as a function of gate voltage for the two peaks. The second peak disappears at the ΔV_g^{DC} value indicated by an arrow. In (C), V_g^{AC} is 0.5 mV, and V_{SD}^{AC} is 0.1 mV. In (D), V_g^{AC} is 0.255 mV, and V_{SD}^{AC} is 0.02 mV. (E and F) Measured and calculated effective quality factor as a function of V_g^{AC} for the second peak at the Coulomb-blockade conductance peak. V_{SD}^{AC} is 0.1 mV in (E) and 0.02 mV in (F). Other parameters used in the calculations are given in (13). The inset shows the calculated vibration amplitude and mixing current as functions of the driving frequency.



only appears when looking at the I_{mix} signal, its high Q is only an effective quality factor, but it is useful for applications that, for instance, rely on precise measurements of the resonance frequency (21).

These results show that the coupling between the mechanics and the electron transport can be very strong in SWNTs. In comparison, experiments on microfabricated semiconductor resonators, which are coupled to metal single-electron transistors, have not shown any oscillations of f_0 and Q (22). This difference probably arises from the much greater mass of these resonators as compared with those of SWNTs, so that they are much less sensitive to the motion of individual tunneling electrons. A way to quantify the coupling is by looking at the damping rate caused by the interaction between the mechanics and the electron transport, $\gamma_{e-ph} = 2\pi f_0/Q$. Damping rate can be useful for the evaluation of quantum electromechanics phenomena in analogy to laser cooling of, for example, trapped ions (23). We have γ_{e-ph} as high as 3×10^6 Hz for nanotubes, which compares with $\gamma_{e-ph} \sim 0.7$ Hz for macroscopic atomic force microscopy cantilevers (24). As for single-electron transistors that are superconducting, γ_{e-ph} is expected to be enhanced (15). In this case, however, γ_{e-ph} measured on microfabricated resonators is below 5×10^4 Hz (12).

The strong coupling in nanotubes holds promise for various applications. We have shown that it allows for a widely tunable nonlinearity of the resonator dynamic. Nonlinearity of the motion is useful for sensitivity improvement of mass and force sensing (25), mechanical signal amplification (26, 27), noise squeezing (28), study of quantum behaviors of macroscopic systems (29), mechanical microwave computing (30), or energy harvesting via vibration-to-electricity conversion (31). We emphasize that the nonlinearity in nanotubes can be tuned by an electronic means (by changing V_g^{DC}), which is convenient for practical use. In comparison, the non-

linearity in previously studied nanoresonators comes from purely mechanical effects (which can be described by the Duffing equation). There, the nonlinearity can be modified by using strain, but this is complicated to do experimentally. The strong coupling in nanotubes could also be used for cooling mechanical oscillations to the ground state (32), which would open the possibility to study nonclassical states at a mesoscopic scale. The ability to electrically tune the coupling (with V_g^{DC}) would then be very useful for quantum manipulation.

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- We took $C_{\text{dot}} = 57$ aF from Fig. 2A. Dissipation caused by He gas used to thermalize the resonator was included by calculating the total quality factor as $(\sum 1/Q_i)^{-1}$. The contribution of Q from He has been measured to be 1035 by means of pumping the gas, which left the temperature stable for a short moment.
- We obtained $C_g'^2/k = 6 \times 10^{-22}$ F²/Nm, which has to be compared with $C_g' \approx 10^{-13} - 10^{-12}$ F/m obtained by using commercial simulators and $k \approx 10^{-4} - 10^{-3}$ N/m from (1). The value of Γ is 3×10^9 s⁻¹, which is somewhat smaller than expected in the quantum regime $\Gamma = 8k_B T G_{\text{max}}/e^2 \approx 9 \times 10^{10}$ s⁻¹ (where G_{max} is the maximum conductance of a Coulomb-blockade peak). In addition, the shape of the measured oscillations of f_0 and Q differs to some extent from what is predicted (Fig. 3). These differences may arise from the fact that the nanotube dot is not perfect (the Coulomb blockade peaks are not fully periodic in V_g^{DC} , and the peak heights are different). Another explanation is that the model that was used is too simple to quantitatively capture the physics (33, 34).
- The oscillation of Q as a function of V_g^{DC} has been observed in a second device. The Coulomb-blockade oscillations of G are, however, less regular than the ones presented here, and so the nanotube probably consists of a series of quantum dots (35).
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The Chemical Structure of a Molecule Resolved by Atomic Force Microscopy

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Resolving individual atoms has always been the ultimate goal of surface microscopy. The scanning tunneling microscope images atomic-scale features on surfaces, but resolving single atoms within an adsorbed molecule remains a great challenge because the tunneling current is primarily sensitive to the local electron density of states close to the Fermi level. We demonstrate imaging of molecules with unprecedented atomic resolution by probing the short-range chemical forces with use of noncontact atomic force microscopy. The key step is functionalizing the microscope's tip apex with suitable, atomically well-defined terminations, such as CO molecules. Our experimental findings are corroborated by ab initio density functional theory calculations. Comparison with theory shows that Pauli repulsion is the source of the atomic resolution, whereas van der Waals and electrostatic forces only add a diffuse attractive background.

Noncontact atomic force microscopy (NC-AFM), usually operated in frequency-modulation mode (1), has become an important tool in the characterization of nano-

structures on the atomic scale. Recently, impressive progress has been made, including atomic resolution with chemical identification (2) and measurement of the magnetic exchange force

with atomic resolution (3). Moreover, lateral (4) and vertical (5) manipulation of atoms and the determination of the vertical and lateral forces during such manipulations (6) have been demonstrated. Striking results have also been obtained in AFM investigations of single molecules. For example, atomic resolution was achieved on single-walled carbon nanotubes (SWNTs) (7, 8), and the force needed to switch a molecular conformation was measured (9).

However, the complete chemical structure of an individual molecule has so far not been imaged with atomic resolution. The reasons that make AFM investigations on single molecules so challenging are the strong influence of the exact

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atomic composition and the geometry of the tip, as well as the relatively low stability of the system, which can result in unintentional lateral or vertical manipulation of the molecule during imaging. As will be shown below, both problems can be solved by preparing a well-defined tip by deliberately picking up different atoms and molecules with the tip apex. The exact knowledge of the tip termination also facilitates quantitative comparison with first-principles calculations, which is essential for understanding the nature of the tip-sample interaction.

To benchmark AFM resolution on molecules, we investigated pentacene ($C_{22}H_{14}$, Fig. 1A), a well-studied linear polycyclic hydrocarbon consisting of five fused benzene rings. State-of-the-art scanning tunneling microscopy (STM) studies of pentacene on metal, such as Cu(111) (10), and thin-film insulators, such as NaCl on Cu(111) (11, 12), have been performed recently. On insulating films, STM was used to image the molecular orbitals near the Fermi level, E_F , whereas on metals the molecular orbitals were broadened and distorted because of coupling to the electronic states of the substrate. STM is sensitive to the density of states near E_F , which extends over the entire molecule. This prevents the direct imaging of the atomic positions (or core electrons) in such planar aromatic molecules by STM. In this work, we present atomically resolved AFM measurements of pentacene both on a Cu(111) substrate and on a NaCl insulating film.

For atomic resolution with the AFM, it is necessary to operate in the short-range regime of forces, where chemical interactions give substantial contributions. In this force regime, it is desirable to work with a cantilever of high stiffness with oscillation amplitudes on the order of 1 Å, as pointed out by Giessibl (13). Our low-temperature STM/AFM has its basis in a qPlus sensor design (14) and is operated in an ultrahigh vacuum at a temperature of 5 K. The high stiffness of the tuning fork [spring constant $k_0 \approx 1.8 \times 10^3$ N/m (15), resonance frequency $f_0 = 23,165$ Hz, and quality factor $Q \approx 5 \times 10^4$] allows stable operation at oscillation amplitudes down to 0.2 Å. A metal tip (16) was mounted on the free prong of the tuning fork, and a separate tip wire (which is insulated from the electrodes of the tuning fork) was attached to measure the tunneling current (17). The bias voltage V was applied to the sample.

Modification of the STM tip apex is known to have a profound influence on the achievable image resolution (10, 11, 18, 19). We explored the effects of controlled atomic-scale modification of the AFM tip and show that suitable tip termination results in dramatically enhanced atomic scale contrast in NC-AFM imaging. We imaged pentacene molecules (Fig. 1A) in STM (Fig. 1B) and AFM (Fig. 1, C and D) modes on Cu(111) by using a CO-terminated tip. For these measurements, a CO molecule was deliberately picked up with the tip (16), which led to an increased resolution in the AFM mode (see below). From previous investigations, it is known that the CO molecule is

adsorbed with the carbon atom toward the metal tip (18, 19).

The CO molecule slightly affects the STM image, and several faint maxima and minima are visible because of the interaction of the CO with the pentacene orbitals, similar to the effect of a pentacene-modified tip (10). The AFM images (Fig. 1, C and D) were recorded in constant-height mode; that is, the tip was scanned without z feedback parallel to the surface while the frequency shift Δf was being recorded (16). In this and all of the following measurements, the tip height z is always given with respect to the STM set point over the substrate. The use of constant-height operation was critical because it allowed stable imaging in the region where Δf is a nonmonotonic function of z . In the AFM images (Fig. 1, C and D), the five hexagonal carbon rings of each pentacene molecule are clearly resolved.

We observed local maxima of $\Delta f(x, y)$ above the edges of the hexagons, near the carbon atom positions, and minima above the centers of the carbon rings (hollow sites), in concordance to the measurements on SWNTs (7). Even the carbon-hydrogen bonds are imaged, indicating the positions of the hydrogen atoms within the pentacene molecule. Additionally, each molecule is surrounded by a dark halo.

To demonstrate that imaging conditions are also stable for the case of organic molecules on insulators, we used a thin insulating layer [NaCl(2 ML)/Cu(111)], that is, two atomic layers of NaCl on Cu(111)] as substrate (Fig. 2). Furthermore, to study the influence of the tip termination, we performed measurements with different atomic modifications of the tip apex. In addition to the Ag- (Fig. 2A) and CO-terminated (Fig. 2B) tips, we also recorded Δf images with

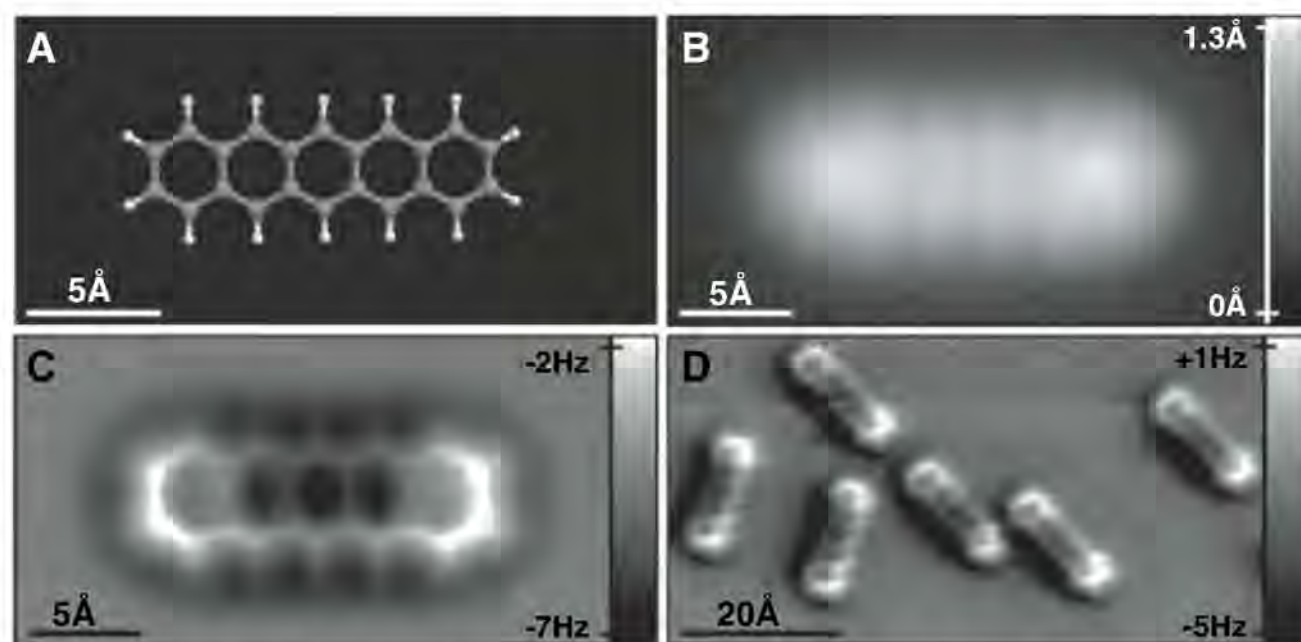


Fig. 1. STM and AFM imaging of pentacene on Cu(111). (A) Ball-and-stick model of the pentacene molecule. (B) Constant-current STM and (C and D) constant-height AFM images of pentacene acquired with a CO-modified tip. Imaging parameters are as follows: (B) set point $I = 110$ pA, $V = 170$ mV; (C) tip height $z = -0.1$ Å [with respect to the STM set point above Cu(111)], oscillation amplitude $A = 0.2$ Å; and (D) $z = 0.0$ Å, $A = 0.8$ Å. The asymmetry in the molecular imaging in (D) (showing a “shadow” only on the left side of the molecules) is probably caused by asymmetric adsorption geometry of the CO molecule at the tip apex.

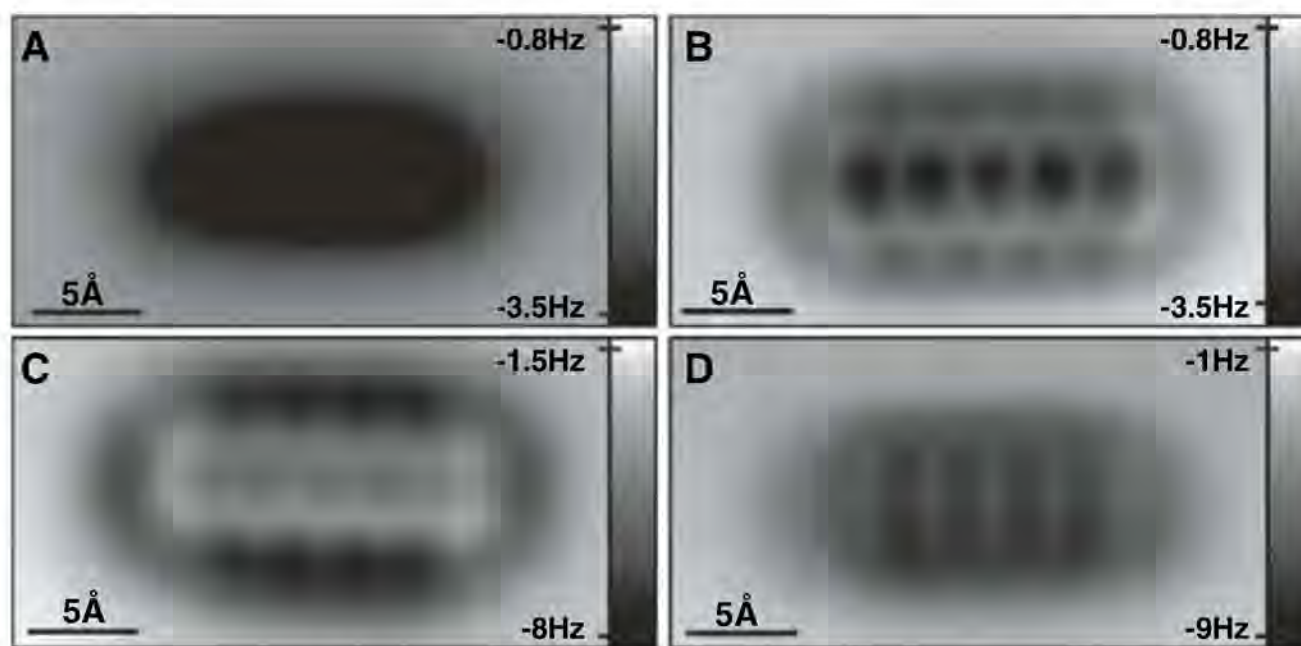


Fig. 2. Constant-height AFM images of pentacene on NaCl(2ML)/Cu(111) using different tip modifications (16). (A) Ag tip, $z = -0.7$ Å, $A = 0.6$ Å; (B) CO tip, $z = +1.3$ Å, $A = 0.7$ Å; (C) Cl tip, $z = -1.0$ Å, $A = 0.7$ Å; and (D) pentacene tip, $z = +0.6$ Å, $A = 0.5$ Å. The z values are given with respect to a STM set point of $I = 2$ pA, $V = 200$ mV above the NaCl(2 ML)/Cu(111) substrate.

tips modified with Cl (Fig. 2C) and pentacene (Fig. 2D) (16). For each tip, the tip height z was minimized; that is, decreasing z further by a few 0.1 Å resulted in unstable imaging conditions,

usually leading to the molecule being laterally displaced or picked up by the tip. Comparing the different tips, we see that their atomic termination is crucial for the contrast observed above the

molecule. The highest lateral resolution was observed with CO-modified tips, and the contrast above pentacene is similar to that in the measurements on Cu(111). With metal-terminated tips

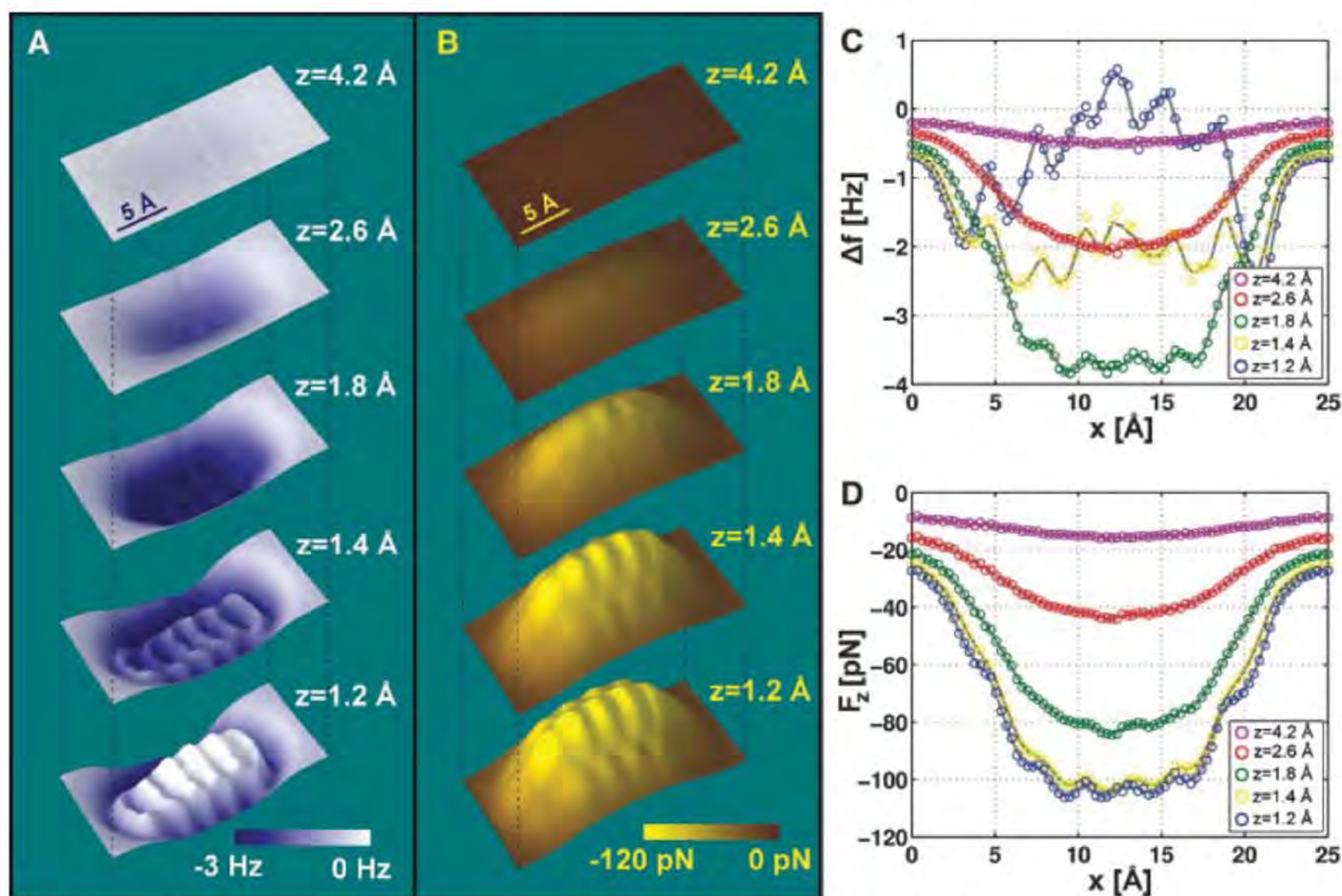


Fig. 3. Maps of measured frequency shift Δf (A) and extracted vertical force F_z (B) at different tip heights z . Corresponding line profiles of Δf (C) and F_z (D) along the long molecular axis. The data shown are part of a complete three-

dimensional force field that has been measured in a box of 25 Å by 12.5 Å by 13 Å above a pentacene molecule (16). The z values are given with respect to a STM set point of $I = 2$ pA, $V = 200$ mV above the NaCl(2 ML)/Cu(111) substrate.

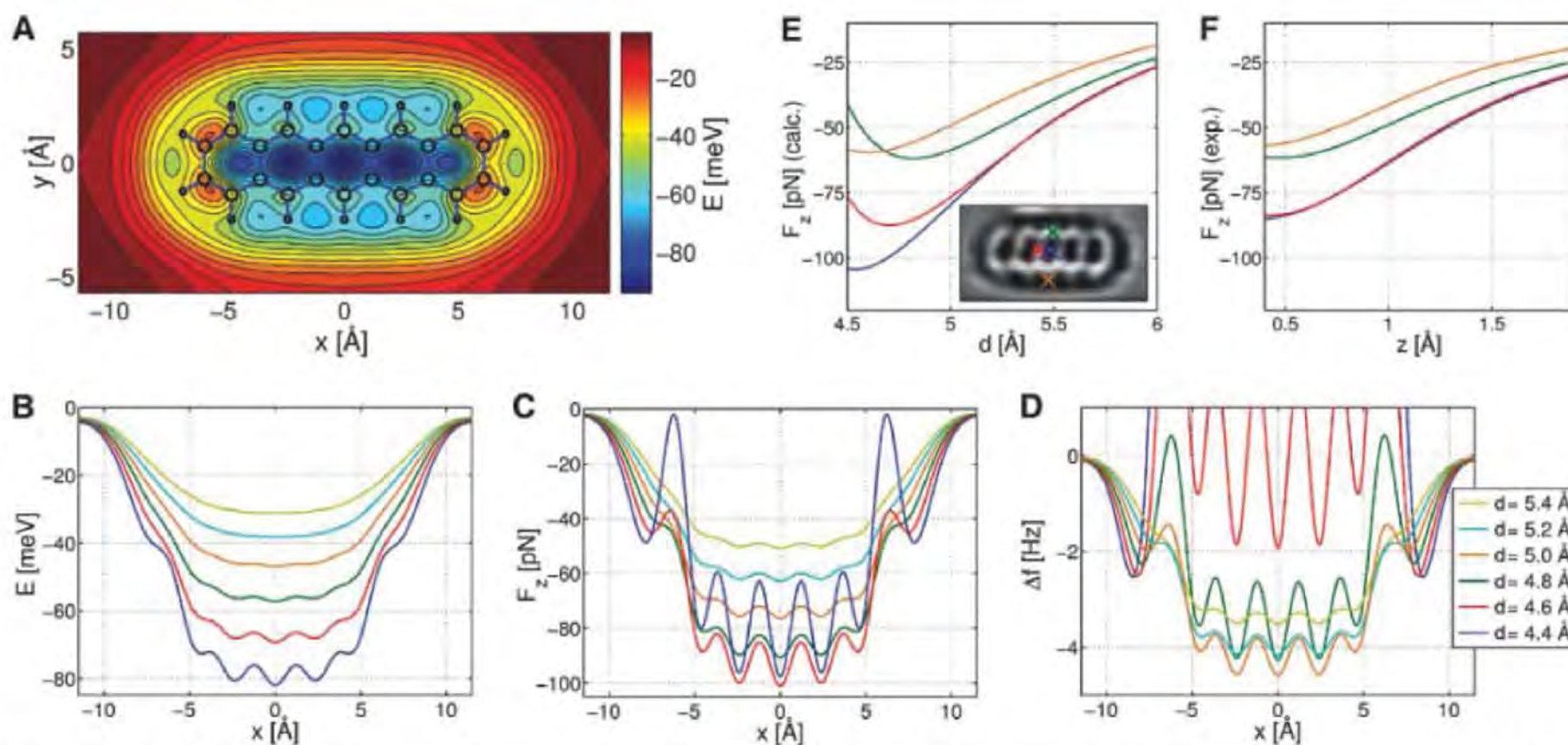


Fig. 4. Calculated energy map (A) for a CO-pentacene distance of $d = 4.5$ Å. Calculated line profiles of the energy (B), the vertical force (C), and Δf (D) above the long molecular axis for different molecular distances. Calculated (E) and measured (F) force-distance curves above different molecular sites: central hollow site (blue), C-C bond of central

ring on long molecular axis (red), C atom of central ring on short molecular axis (green), and H atom on short axis (orange). The inset in (E) shows a measured Δf map with the different molecular sites indicated. In the experimental data, the force components that arise from the metal tip behind the CO molecule have been subtracted (16, 29).

(Ag, Au, or Cu), the molecule would always be manipulated (usually being picked up by the tip from NaCl) before the minimum of $\Delta f(z)$ was reached, and no atomic resolution could be observed in AFM measurements. The Cl termination yielded a contrast similar to that of the CO tip. However, in the AFM image acquired with the Cl tip, the minima above the hollow sites are less pronounced and the carbon rings appear smaller in diameter compared with the CO-terminated tip. The pentacene-modified tip gave a completely different contrast compared with all the other tips investigated, which indicates the strong influence of the tip modification.

In the following, we concentrate on the investigation of pentacene on NaCl(2 ML)/Cu(111) with a CO-terminated tip. We describe how the contrast depends on the tip sample distance, quantify the forces acting on the tip, and lastly compare the measurements to density functional theory (DFT) calculations in order to separate the contributions of different forces and understand the origin of the observed contrast. Frequency shift and force versus distance relations were determined by capturing a three-dimensional (x, y, z) field (16, 20, 21) of the frequency shift with 80 by 40 by 3100 data points in a box of 25 Å by 12.5 Å by 13 Å above a pentacene molecule. We extracted the vertical forces $F_z(x, y, z)$ with use of the method of Sader and Jarvis (16, 22). Figure 3A shows the measured frequency shift at different tip heights, and the extracted vertical force is shown in Fig. 3B. Corresponding line profiles along the long molecular axis are given in Fig. 3, C and D, for Δf and F_z , respectively.

For distances greater than $z = 4.2$ Å (top image in Fig. 3A), we recorded only relatively small long-range forces ($F_z < 20$ pN), and the molecule was imaged as a featureless depression (attractive interaction). With decreasing tip height, $\Delta f(z)$ decreased before reaching a minimum (of about -4 Hz) above the molecular center at $z \approx 1.8$ Å. Near the minimum of $\Delta f(z)$, we started observing corrugation on the atomic scale (Fig. 3C). When we decreased z further, Δf increased again and finally became even positive over some parts of the molecule at $z = 1.2$ Å. The lateral contrast of Δf generally increased with decreasing tip height (Fig. 3C). At the height at which Δf crossed zero ($z \approx 1.2$ Å), we achieved the highest contrast and lateral resolution with AFM. This is the regime of maximal attractive forces (16). Decreasing the tip height further would result in instabilities and lastly in picking up the molecule by the tip.

In the force maps (Fig. 3B), we likewise observed how the contrast increased with decreasing z . For the smallest accessible tip height, that is, $z = 1.2$ Å, we measured an attractive force of 110 pN above the central carbon ring. Above the positions of the carbon atoms, the absolute values of the forces were smaller (between 60 and 90 pN) (15). These values are comparable to the maximum short-range forces acting on a silicon tip above the hollow sites and the carbon positions of a SWNT,

which were recently measured as 106 pN and 75 pN, respectively (7) (also with a systematic error of about 30%).

To further elucidate the origin of the observed atomic contrast, we carried out DFT calculations (16, 23, 24) with highly optimized plane-wave code CPMD (25). We applied the PBE (Perdew-Burke-Ernzerhof) exchange-correlation functional (26) and used ab initio norm-conserving pseudopotentials (27). Van der Waals (vdW) forces were added semiempirically to the dispersion energy as a contribution proportional to R^{-6} (where R is the atomic distance) (28). We only included the pentacene molecule and the CO molecule in our calculations and neglected both the substrate and the metallic part of the tip. We assumed for the calculations that the CO molecule is perpendicular to the plane of the pentacene molecule, with the oxygen atom pointing toward the pentacene (16).

The calculated interaction energy surface is given in Fig. 4A, and the calculated constant-height line profiles of the energy, the vertical force, and Δf along the central molecular axis are shown in Fig. 4, B, C, and D, respectively. The intermolecular distance d denotes the distance of the CO carbon atom to the plane of the pentacene atoms. Experiment and theory (compare Figs. 3D and 4C) concordantly showed maximal attractive forces on the order of 100 pN above the hollow sites of the pentacene molecule, whereas attractive forces above the C-C bonds were smaller. The difference in the forces measured above these two sites increased with decreasing tip height. In the short-range regime ($d < 5$ Å), differences between calculations and experiment can be observed. In the calculations, sharp peaks appear above the outer C-C bonds ($x = 6.4$ Å and $x = -6.4$ Å in Fig. 4, C and D), which are less pronounced in the measurements (Fig. 3, C and D).

For a comparison between theory and experiment, we have to estimate the contribution of the metallic tip behind the CO molecule that was not included in the calculations. For this purpose, we measured the force acting on a purely metallic tip, then picked up a CO molecule and measured the force again under otherwise identical conditions. The difference of the forces for identical tip positions yielded the estimated contribution from the CO molecule only. We observed that the CO contribution to the force predominates in the relevant regime, whereas the metallic part of the tip contributed only about 30% to the attractive forces and gave no corrugation on the atomic scale (16). Calculated force-versus-distance curves (Fig. 4E) above different molecular sites around the central carbon ring of the molecule are compared with experimental data, with the contribution of the metallic tip subtracted (Fig. 4F). We observe good qualitative agreement between theory and experiment in terms of the maximal values of the force and the relative order in which the maximal attractive forces are reached above the different atomic sites (29). Quantitatively, the agreement is excellent for $d > 5$ Å. In

the short-range regime (4.5 Å $< d < 5$ Å), the calculations overestimate the difference in the forces above the C-C bond and the hollow site. These discrepancies in the short-range region might arise because of the simplifications assumed in the calculations, namely not taking the substrate or the tip behind the CO into account and the semiempirical treatment of vdW forces. Another origin of the discrepancies could be the increasing influence of noise in the experimental data for small z values.

The calculations take into account forces of three different physical origins, namely electrostatic forces, vdW forces, and Pauli repulsive forces. Comparing their contributions to the overall force, we found that the electrostatic forces are small ($\sim 10\%$) compared with the vdW forces. These two contributions to the force show little lateral corrugation on the atomic scale and yield a diffuse attractive potential above the entire molecule, giving rise to the observed dark halo surrounding the molecules in the Δf maps. The origin of the atomic contrast is the Pauli repulsion force, which becomes substantial when regions of high electron density overlap. These regions are concentrated to the atomic positions and to the C-C (and to a lesser extent also to the C-H) bonds in the pentacene molecule and are revealed for sufficiently small tip-sample distances ($d \approx 5$ Å).

We conclude that atomic resolution in NC-AFM imaging on molecules can only be achieved by entering the regime of repulsive forces because the vdW and electrostatic forces only contribute a diffuse attractive background with no atomic-scale contrast. Modifying the tip with suitable atomic or molecular terminations is required to allow the AFM to be operated in this regime while maintaining stable imaging conditions. The tip termination governs the AFM contrast, and exact knowledge of the tip is needed for a detailed interpretation of the force measurements. In the case of the CO-terminated tip, we observed a spectacular enhancement of the atomic-scale contrast and were able to resolve the atomic positions and bonds inside pentacene molecules, precisely revealing the atomic molecular structure. It may also be possible to extract details about intermolecular bonds, for example, bond order and length. Furthermore, we foresee probing the reactivity of different molecular sites with respect to the known molecule or atom at the tip apex. Such investigations could yield detailed insight into chemical reactions and catalysis. Lastly, a combination of NC-AFM with electrostatic force microscopy could be used to investigate single-electron transport and charge distributions in metal-molecule systems on the atomic scale.

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29. The experimental and theoretical distance values z and d could be related to each other, for example, by comparing the position of the force minimum above a certain molecular site. However, the experimental z position of the force minimum varies significantly between measurements with different CO tips (in Fig. 3 the force minimum was already reached at $z \approx 1.2$ Å, whereas in Fig. 4F it is not reached until $z \approx 0.4$ Å). We attribute this to the fact that the tunneling current, and hence the tip height corresponding

to the STM set point, strongly depends on the exact adsorption geometry of the CO molecule at the tip apex. Therefore, we abstain from giving an explicit relation between z and d .

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Supporting Online Material

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Materials and Methods

Figs. S1 to S5

References

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Amplifying the Pacific Climate System Response to a Small 11-Year Solar Cycle Forcing

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One of the mysteries regarding Earth's climate system response to variations in solar output is how the relatively small fluctuations of the 11-year solar cycle can produce the magnitude of the observed climate signals in the tropical Pacific associated with such solar variability. Two mechanisms, the top-down stratospheric response of ozone to fluctuations of shortwave solar forcing and the bottom-up coupled ocean-atmosphere surface response, are included in versions of three global climate models, with either mechanism acting alone or both acting together. We show that the two mechanisms act together to enhance the climatological off-equatorial tropical precipitation maxima in the Pacific, lower the eastern equatorial Pacific sea surface temperatures during peaks in the 11-year solar cycle, and reduce low-latitude clouds to amplify the solar forcing at the surface.

It has long been noted that the 11-year cycle of solar forcing is associated with various phenomena in Earth's climate system, in both the troposphere and stratosphere (1–9). Because the amplitude of the solar cycle (solar maximum to solar minimum) is relatively small, about 0.2 W m^{-2} globally averaged (10), and the observed global sea surface temperature (SST) response of about 0.1°C would require more than 0.5 W m^{-2} (11), there has always been a question regarding how this small solar signal could be amplified to produce a measurable response.

Postulated mechanisms that could amplify the relatively small solar forcing signal to produce such responses in the troposphere include changes in clouds in the troposphere caused by galactic cosmic rays, or associated global atmospheric electric circuit variations, though neither has been plausibly simulated in a climate model. However, there are two other plausible mechanisms, though each has not yet produced a modeled response of the magnitude seen in the observations. The first involves a "top down" response of stratospheric ozone to the ultraviolet (UV) part of the solar spectrum that varies by a few percent. Peaks in solar forcing cause the enhanced UV radiation, which stimulates additional stratospheric ozone production and UV absorption, thus warming that layer differentially with respect to latitude. The anomalous temperature gradients provide a positive feedback through wave motions to amplify the original solar forcing. The changes in the stratosphere modify tropical tropospheric circulation and thus contribute to an enhancement and poleward expansion of the tropical precipitation

maxima (5, 12–16). The first demonstration of the top-down mechanism in a modeling study showed a broadening of the Hadley cells in response to enhanced UV that increased as the solar-induced ozone change was included (17).

A second "bottom up" mechanism that can magnify the response to an initially small solar forcing involves air-sea coupling and interaction with incoming solar radiation at the surface in the relatively cloud-free areas of the subtropics. Thus, peaks in solar forcing produce greater energy input to the ocean surface in these areas, evaporating more moisture, and that moisture is carried by the trade winds to the convergence zones where more precipitation occurs. This intensified precipitation strengthens the Hadley and Walker circulations in the troposphere, with an associated increase in trade wind strength that produces greater equatorial ocean upwelling and lower equatorial SSTs in the eastern Pacific, a signal that was first discovered in observational data (1, 2). The enhanced subsidence produces fewer clouds in the equatorial eastern Pacific and the expanded subtropical regions that allow even more solar radiation to reach the surface to produce a positive feedback (18, 19). Dynamical air-sea coupling produces a transition to higher eastern equatorial SSTs a couple of years later (20, 21). There is observational evidence for a strengthened Hadley circulation in peak solar forcing years associated with intensified tropical precipitation maxima, a stronger descending branch in the subtropics, and a stronger ascending branch in the lower latitudes (3); a poleward expansion of the Hadley circulation in peak solar years, with stronger ascending motions at the edge of the rising branch, as well as a stronger Walker circulation with enhanced upward motions in the tropical western Pacific connected to stronger descending motions in the tropical eastern Pacific (7); and enhanced summer season off-equatorial climatological monsoon precipitation over India (6, 22). This cold event-like response to peak solar forcing is different from cold events (also known as La Niña events)

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in the Southern Oscillation in that, among other things, zonal wind anomalies in the stratosphere are opposite in sign (23).

The top-down stratospheric UV ozone mechanism and the bottom-up coupled air-sea response mechanism have been simulated separately in various climate model experiments that seem to

produce elements of the observations, with some evidence of enhancement of off-equatorial tropical precipitation maxima, and a poleward expansion of the Hadley circulation (5, 12, 13, 16, 19, 24, 25). A critical weakness of these studies is that either alone does not seem to produce the signals of the amplitude seen in the observations, and no model

simulation has been able to simultaneously include both mechanisms with time-evolving 11-year solar cycle forcing. Up until now it was speculated that these two mechanisms could be additive and thus amplify the small solar forcing signal to produce responses more comparable in amplitude to those seen in observations (19). Here

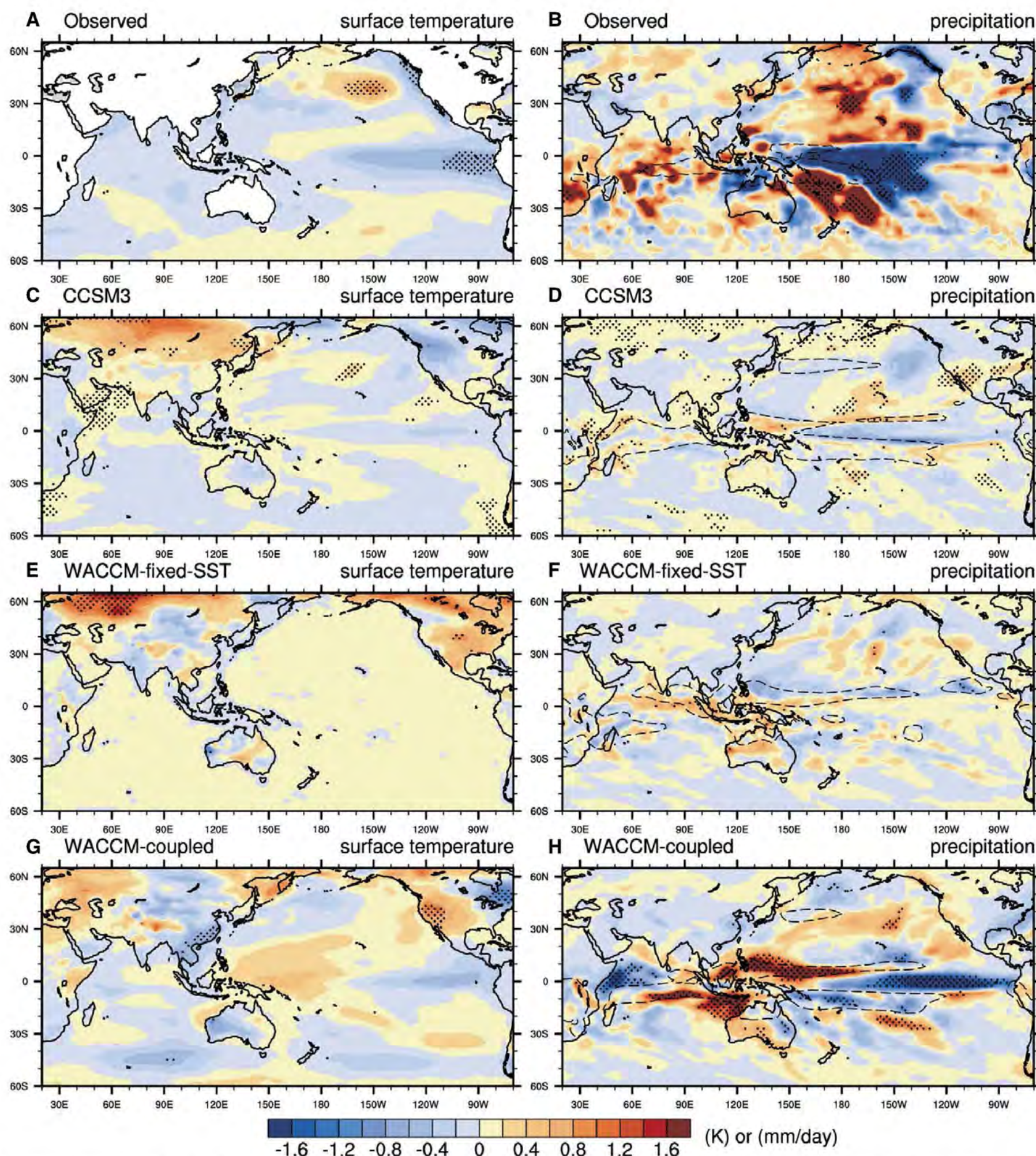


Fig. 1. Composite averages for DJF for peak solar years. (A) Observed SSTs for 11 peak solar years (2) ($^{\circ}\text{C}$). (B) Same as (A) except for precipitation for three available peak solar years (2) (mm day^{-1}). (C) Same as (A) except for CCSM3 average of five ensemble members for 20th-century climate (19) ($^{\circ}\text{C}$). (D) Same as (C) except for precipitation (19) (mm day^{-1}). (E) Same as (A) except for WACCM with specified nonvarying SSTs, for 10 peak solar

years. (F) Same as (E) except for precipitation (mm day^{-1}). (G) Same as (A) except for WACCM coupled to the dynamical ocean, land, and sea ice components of CCSM3, for 11 peak solar years ($^{\circ}\text{C}$). (H) Same as (G) except for precipitation (mm day^{-1}). Stippling indicates significance at the 5% level, and dashed lines indicate position of climatological precipitation maxima.

we use several related climate model versions wherein we can include both mechanisms separately (an atmospheric model with no stratospheric dynamics or chemistry coupled to ocean, land, and sea ice; an atmospheric model with stratospheric dynamics and ozone chemistry driven by specified SSTs and sea ice) and then combine them (the atmospheric model with stratospheric dynamics and ozone chemistry coupled to the ocean, land, and sea ice) to test if they can, indeed, amplify the climate system response to solar forcing to produce responses of the magnitude seen in the observations.

Composite observations for peaks in the 11-year solar cycle [how peak years are chosen is described in the supporting online material (SOM); all anomalies are computed for composite peak solar years minus climatology; anomalies for peak solar years minus solar minimum years would be about twice as large] show negative SST anomalies in the eastern equatorial Pacific of about -0.8°C (2), and poleward-shifted and intensified climatological precipitation maxima in the Pacific [the Intertropical Convergence Zone (ITCZ) north of the equator and South Pacific Convergence Zone (SPCZ) south of the equator] with anomalies greater than $+1\text{ mm day}^{-1}$, with reduced precipitation (and clouds) over the anomalously low SSTs of about the same magnitude but opposite sign, amounting to changes of $\sim 10\%$ in the ITCZ and SPCZ (Fig. 1, A and B).

The first climate model used here is a global coupled climate model [the Community Climate System Model version 3 (CCSM3); see SOM] that has coupled components of atmosphere, ocean, land, and sea ice. It does not have a resolved stratosphere and no interactive ozone chemistry, so the CCSM3 includes only the bottom-up coupled air-sea mechanism. This model is run for five realizations of 20th-century climate with anthropogenic and natural forcings (including the 11-year solar cycle; see SOM). An ensemble average composite for northern winter [December-January-February (DJF)] of 11 peak solar years shows that this model simulates the coupled air-sea response mechanism at the surface in the tropical Pacific with relatively weak negative SST anomalies in the equatorial eastern Pacific (about -0.2°C or a fourth the size of the observed) and an enhanced and poleward-shifted ITCZ and SPCZ precipitation maxima with anomalies of around $+0.3\text{ mm day}^{-1}$ (about a third the size of the observations; Fig. 1, A and B), with negative precipitation anomalies over the anomalously cold water in the eastern equatorial Pacific.

The second climate model, a version of the Whole Atmosphere Community Climate Model (WACCM; see SOM) is a global atmospheric model run with climatological SSTs and changes in solar variability (other external forcings are held constant). It has no dynamical coupled air-sea interaction, but does include a resolved stratosphere, fully interactive ozone chemistry that can respond to the UV part of the solar forcing, and thus should include the top-down stratospheric

ozone mechanism. The model is run with 11 11-year solar cycles, and composites are formed for the DJF season for peak solar years. Because the SSTs are specified and are the same every year, there is no SST signal in response to solar forcing by design, but there is an enhancement of the tropical climatological precipitation maxima, with increases of up to 0.4 mm day^{-1} in the tropical eastern Indian and western Pacific, and positive anomalies in the ITCZ and SPCZ (Fig. 1, E and F). These precipitation changes are still about a factor of 3 smaller than observed and are more concentrated in regions where there are largest values of climatological precipitation in the equatorial Indian and western Pacific Ocean regions because there is no dynamical ocean response in the tropical Pacific.

The third climate model uses the atmospheric component (WACCM) from the second model above, but it is coupled to the dynamical ocean, land, and sea ice modules in CCSM3. Therefore, this global coupled climate model should include both the top-down stratospheric ozone mechanism and the bottom-up coupled air-sea interaction mechanism. There are negative SST anomalies in the equatorial eastern Pacific of greater than -0.6°C (Fig. 1G), much closer to the observed values of -0.8°C . These are a factor of 3 greater than the CCSM3 that includes only the bottom-up air-sea coupled mechanism. The precipitation anomalies show an enhanced ITCZ and SPCZ in the tropical Pacific and strengthened precipitation in the tropical Indian Ocean with values greater than $+1\text{ mm day}^{-1}$ (Fig. 1H), with reduced precipitation over the anomalously cold water in the eastern equatorial Pacific comparable to the ob-

served anomalies (Fig. 1B). Thus, these models indicate that each mechanism acting alone [the bottom-up surface coupled air-sea mechanism in CCSM (Fig. 1, C and D) and the top-down stratospheric ozone mechanism in WACCM (Fig. 1, E and F)] can produce a weak signature of the observed enhancement of the tropical precipitation maxima, but when both act in concert [in the coupled version of WACCM (Fig. 1, G and H)], the two mechanisms work together to produce climate anomalies much closer to the observed values (Fig. 1, A and B), thus amplifying the relatively small solar forcing to produce significant SST and precipitation anomalies in the tropical Indo-Pacific region. This highlights the importance of stratospheric processes working in conjunction with coupled processes at the surface.

Zonal mean precipitation anomalies averaged around the globe for the observations show the enhanced and poleward-shifted off-equatorial precipitation maxima in the tropics with values of greater than $+0.2\text{ mm day}^{-1}$ (on the order of 20%), with reduced precipitation in the equatorial region of more than -0.4 mm day^{-1} (Fig. 2). The CCSM3 with only the bottom-up coupled air-sea interaction mechanism shows a weak enhancement of the off-equatorial zonal mean precipitation maxima of less than 0.1 mm day^{-1} , with reduced near-equatorial precipitation of just more than -0.1 mm day^{-1} , a factor of about 4 less than the observations. The WACCM run with specified SSTs (with only the top-down mechanism) shows more of an enhancement of zonal mean precipitation in the equatorial regions of about $+0.15\text{ mm day}^{-1}$, mainly from the greater climatological precipitation in the tropical western

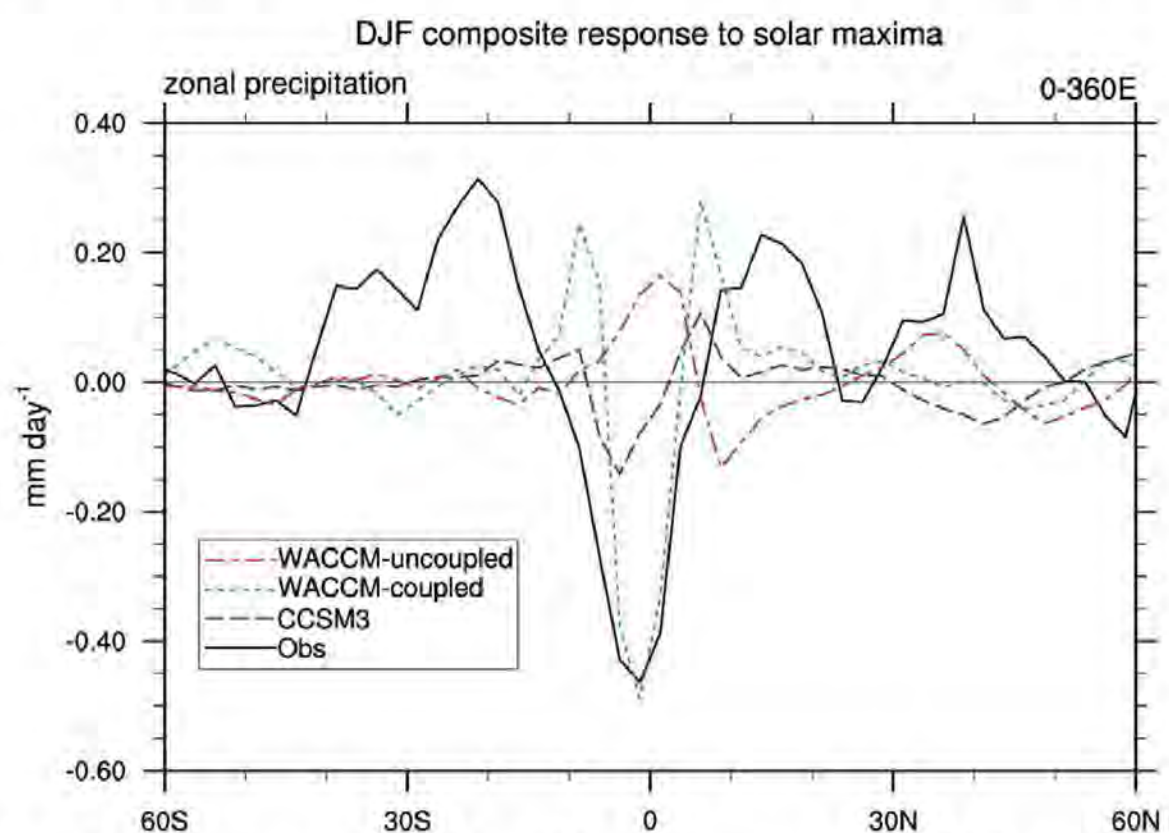


Fig. 2. Global zonal mean precipitation for DJF for 3 peak solar years available in the observations (January–February, black solid line); WACCM uncoupled run with specified SSTs for 11 peak solar years (red dash-dot line); WACCM coupled to the dynamical ocean, land, and sea ice components of CCSM3 for 11 peak solar years (green dashed line); and CCSM3 for five-member ensemble mean of 11 peak solar year composites (blue dashed line). Enhanced off-equatorial tropical precipitation in CCSM3 and WACCM-coupled near 10°N and 10°S are shifted closer to the equator than the observed peaks near 15°N and 20°S because of a systematic error in the coupled models of a too-narrow equatorial Pacific cold tongue.

Pacific and eastern Indian Ocean regions (Fig. 1F). However, the WACCM coupled to the CCSM3 ocean, land, and sea ice components (with both top-down and bottom-up mechanisms) produces an off-equatorial enhancement of zonal mean precipitation more than $+0.2 \text{ mm day}^{-1}$, values close to the observed off-equatorial maxima, with a suppression of equatorial zonal mean precipitation that is about the same as the observed values of about $-0.45 \text{ mm day}^{-1}$. The coupled WACCM positive zonal mean tropical precipitation anomalies are located closer to the equator compared to the more poleward-shifted observational ones due to the width of the negative SST anomalies in the equatorial Pacific. The observations show negative SST anomalies of amplitude greater than -0.2°C extending from the Date Line to the west coast of South America, and from about 10°N to 10°S (Fig. 1A), whereas the CCSM3 (Fig. 1C) and coupled WACCM (Fig. 1G) have a much narrower latitudinal extent of negative SST anomalies of that magnitude, ranging from only about 5°N to 5°S . This is consistent with a more narrow extent of the climatological eastern Pacific cold tongue that is typical of this class of global coupled climate model (26). Therefore, the dynamically coupled interactions in the equatorial eastern Pacific, whereby the trade winds are strengthened in response to the greater upward vertical motions in the intensified precipitation maxima of the ITCZ and SPCZ, produce stronger upwelling of cold water closer to the equator, and thus enhanced ITCZ and SPCZ precipitation maxima closer to the equator in the CCSM3 and coupled WACCM versions compared to the observations (Fig. 2).

The coupled air-sea response mechanism (18, 19) involves locally large net solar flux at the surface compared to the global average, with some values exceeding 1 W m^{-2} locally in the relatively cloud-free areas of the subtropical Pacific in CCSM3 (19). The tropical Pacific (30°N to 30°S , 150°E to 90°W) average net solar radiation at the surface of $+1.03 \text{ W m}^{-2}$ for the coupled WACCM is associated with an overall warming of $+0.06^\circ\text{C}$ [the negative areas in the eastern equatorial Pacific are compensated by the positive SST increase in the off-equatorial regions (Fig. 1G)]. This is comparable to the observed global mean surface temperature anomaly of $+0.07^\circ\text{C}$ for DJF (14) and an estimate of solar maximum to solar minimum variability of observed SSTs for the tropical Pacific area in the year after peak solar forcing of about $+0.04^\circ\text{C}$ (fig. S3). However, there is a net solar radiation anomaly at the surface over the tropical Pacific of only $+0.1 \text{ W m}^{-2}$ for WACCM run with specified nonvarying SSTs, and $+0.21 \text{ W m}^{-2}$ for CCSM3. To achieve an average warming of the ocean surface layer approaching 0.1°C , there must be a net surface heat flux of more than 0.5 W m^{-2} (11, 13). The globally averaged top-of-atmosphere solar forcing for peak solar years compared to solar minimum years is about 0.2 W m^{-2} (10). At the surface at a given location, the diurnal cycle must be taken into account, thus producing about a

factor of 2 greater than the global average. All else being equal, there could be about 0.4 W m^{-2} net solar radiation at the surface in regions of the tropics and subtropics for solar maximum to minimum, for an amplitude of 0.2 W m^{-2} . However, for the tropical Pacific, the coupled WACCM version with both solar response mechanisms is producing more than twice that net solar radiation at the surface, with an attendant factor of roughly 4 for surface temperature increase. Despite uncertainties associated with observed surface fluxes derived from reanalysis data (27), global tropic-averaged (20°N to 20°S) latent plus sensible heat flux associated with the top-down mechanism could be close to 0.5 W m^{-2} (28). The only present model versions that approach that number are coupled WACCM with 0.2 W m^{-2} for the first half of the peak solar year composite for downward latent plus sensible heat flux, and WACCM forced with fixed SSTs with 0.4 W m^{-2} for the first half of the year after peak solar forcing. CCSM3 without the top-down mechanism shows latent plus sensible heat fluxes of near zero for those time periods. However, for coupled WACCM that includes the bottom-up mechanism, the tropic-averaged net solar radiation at the surface for that time period is 0.3 W m^{-2} , roughly three times the amplitude of the solar cycle forcing at the top of the atmosphere, whereas for WACCM forced with fixed SSTs that value is near zero.

This additional amplification of net solar radiation at the surface in coupled WACCM is coming from cloud feedbacks involved with the changes in tropical atmospheric circulation. As the Walker and Hadley cells intensify with their greater vertical motions associated with the enhanced precipitation maxima, there is also greater subsidence in the eastern equatorial Pacific and subtropical Pacific, further reducing cloud amount there and allowing more solar radiation to reach the surface. Both CCSM3 and WACCM run with specified SSTs show some local reductions in cloud amount over regions of the subtropics and eastern equatorial Pacific where there are decreases in precipitation. But in the coupled WACCM, where both mechanisms are active, there is a reduction of tropical Pacific cloud amount of about 2%. Thus, the solar forcing signal is amplified not only by coupled air-sea dynamics and positive ozone-temperature-wind feedbacks, but also by cloud feedbacks in the tropical-subtropical Pacific region. The net effect of these dynamical responses to peaks in solar forcing is that there are negative SST and precipitation anomalies in the tropical Pacific (stronger Walker circulation means stronger trade winds and consequently a coupled dynamical response of greater upwelling of cool water in the equatorial eastern Pacific), and associated cloud feedbacks that provide about a factor of 2 amplification of the net solar forcing at the surface to produce an SST increase of about 0.06°C averaged over the tropical Pacific.

The top-down stratospheric ozone mechanism in the WACCM model versions involves greater absorption of UV solar energy that produces in-

creased ozone in the stratosphere of about 2% with a warming of the stratosphere above 50 hPa [about $+0.3^\circ$ to $+0.4^\circ\text{C}$ at 1 hPa averaged over the DJF and March-April-May seasons of the peak solar year for coupled WACCM and WACCM with fixed SSTs, compared to estimates of observed values of $+0.8^\circ\text{C}$ in the lower stratosphere (28)] that is not present in the CCSM3 without ozone chemistry (shown for December of peak solar years in fig. S1, A, D, and G). These changes in temperature structure in the WACCM model versions produce decreases of westerly-component wind in the lower stratosphere north of about 40°N and increases near 30°N and 30°S (fig. S1, E and H), changes in wave-propagation characteristics (14), and changes in vertical velocity. There is enhanced downward motion in the troposphere near 35°N and 35°S , and intensified upward motion in the tropics (fig. S1, F and I) that is stronger and extends farther into the lower stratosphere than in the CCSM3 (fig. S1C).

The nature of the changes in vertical motion in the tropical troposphere relate in part to the coupled response at the surface. WACCM with specified SSTs shows stronger vertical motion near the equator (fig. S1F) with the enhanced precipitation there (Fig. 2). WACCM coupled to the dynamical ocean with negative equatorial Pacific SST anomalies (Fig. 1) has greater vertical motion in the ITCZ and SPCZ with descending motion near the equator (fig. S1I). There is also a poleward shift of the subtropical jet in the troposphere in both WACCM versions that is strongest with the dynamical ocean coupling (positive zonal wind anomalies near 40°N and 40°S and negative anomalies near 20°N and 20°S in the upper troposphere near 200 hPa; fig. S1, E and H). The evolution to the following June also shows the strongest response in the WACCM coupled to the dynamical ocean, with warming in the troposphere of around $+0.2^\circ\text{C}$ (fig. S2G) that is comparable to an observational estimate of about the same amount (28). This goes along with the transition to higher equatorial Pacific SSTs following peak solar years (fig. S3), intensified upward vertical motion near 10° to 20°N (fig. S2K) associated with stronger monsoon precipitation as seen in observations [e.g., (1, 6)], and anomalous descent near 5°S . Further discussion of the temperature response due to solar forcing is given in the SOM.

An extensive set of model simulations that included a nondynamic mixed-layer ocean (25) produced similar responses to the present coupled WACCM experiments [maximum ozone increase of about 2% near 10 hPa, stratospheric warming with an amplitude of about 0.3°C (or 0.6°C solar maximum minus minimum), climatological off-equatorial precipitation enhancement, and an expansion of the Hadley circulation]. However, the inclusion of a dynamical ocean component in the present experiments simulates the cold event-like response and associated cloud feedbacks at peaks of solar forcing due to coupled atmosphere-ocean dynamics that the nondynamic ocean could not produce (25).

The role of the Quasi-biennial Oscillation (QBO) in the response to solar forcing has been noted in earlier studies (3). A set of experiments with the two WACCM model versions with a prescribed QBO has been carried out, and results from those experiments will be presented in a subsequent paper. However, the results for the climate system response to solar forcing are qualitatively similar to those presented here without the QBO, but the prescribed QBO shows improvements in the stratospheric response compared to observations. Though the solar-forced eastern equatorial SST anomalies shown here are about half the amplitude of those associated with the El Niño–Southern Oscillation, they are relevant for understanding decadal time-scale variability in the Pacific. This response also cannot be used to explain recent global warming because the 11-year solar cycle has not shown a measurable trend over the past 30 years (10).

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Supporting Online Material

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Methods

Figs. S1 to S3

References

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Good Genes and Good Luck: Ammonoid Diversity and the End-Permian Mass Extinction

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The end-Permian mass extinction removed more than 80% of marine genera. Ammonoid cephalopods were among the organisms most affected by this crisis. The analysis of a global diversity data set of ammonoid genera covering about 106 million years centered on the Permian-Triassic boundary (PTB) shows that Triassic ammonoids actually reached levels of diversity higher than in the Permian less than 2 million years after the PTB. The data favor a hierarchical rather than logistic model of diversification coupled with a niche incumbency hypothesis. This explosive and nondelayed diversification contrasts with the slow and delayed character of the Triassic biotic recovery as currently illustrated for other, mainly benthic groups such as bivalves and gastropods.

During the Paleozoic and Mesozoic, ammonoids represented an abundant, highly diversified, and geographically widespread group of marine cephalopods. As a major component of open marine biotas, the diversity and evolution of these shelly mollusks closely record the succession of Paleozoic to Mesozoic global changes (1–3). The Permian is characterized by four major, slowly evolving clades of ammonoids

showing a protracted, two-step decline during the Late Permian (Capitanian and Changhsingian extinctions) (4). Only three known ammonoid genera among Ceratitida survived the Permian-Triassic boundary (PTB); with very few exceptions, Triassic ammonoids are usually found to root into a single genus and are therefore interpreted as a monophyletic clade (1, 3, 5, 6). Their extinction selectivity and patterns of recovery have been addressed through changes of morphological diversity (7–9), taxonomic richness, endemism, and biogeographical distribution viewpoints (1, 2). One problem has been a lack of absolute age calibration of evolutionary trends across the PTB. We have used diversity analyses combined with recently published radiometric ages (10) to show that Triassic ammonoids diversified explosively in the first million years after the PTB.

It has usually been assumed that the end-Permian mass extinction affected ecological as-

semblages so deeply that the postcrisis biotic recovery spanned the entire Early Triassic [~5 million years (My) (11)], if not more (12–14). To test this scenario, we constructed a global taxonomic data set at the generic level, from the Late Carboniferous [Kasimovian, 307 million years ago (Ma)] to the Late Triassic (Rhaetian, 201.5 Ma). For each time bin, we considered all documented occurrences of ammonoid genera for each major oceanic sedimentary basin. The resulting data table records the occurrence of 860 genera within 77 basins through 25 successive time bins of unequal duration. Paleozoic ammonoid data are independently derived from the last versions of the Goniatic and Ammon databases (15, 16); Triassic ammonoid data are compiled from various sources (10), with the latest published genera and occurrences added in all cases. Due to distinct taxonomic treatments between the two Paleozoic databases, higher generic richness counts per time bin are obtained from Ammon (Fig. 1), but these differences have no consequence on origination and extinction rate estimates (fig. S1). We thus selected the published Goniatic database for further analyses.

For each time bin, we derived the total (observed + inferred; S_{obs}) number of genera and estimated overall generic richness using Chao2 and Jackknife2 nonparametric indices (10) (Fig. 1 and table S1). Due to the nature of the available data (generic occurrences within basins), the close correspondence between S_{obs} and Chao2 and Jackknife2 estimators is strong evidence that most time bins have qualitatively similar structures of observed basin incidences resulting from comparable taxonomical practices and sampling efforts along the analyzed time series. There is no evidence that Triassic time bins contain more genera falsely identified as singletons (i.e., genera spanning only one time bin) than Permian ones. When combined with the

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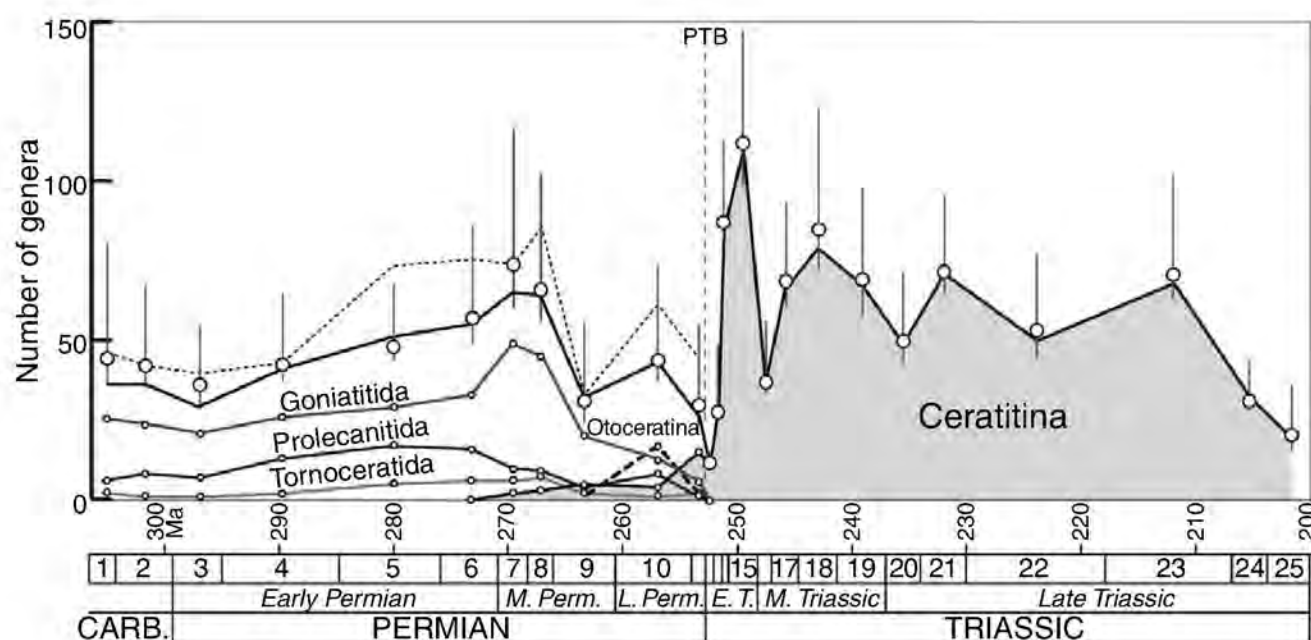


Fig. 1. Total generic richness [S_{obs} ; black bold line, all ammonoids; gray lines, major ammonoid groups; Permian dotted line, alternate data from Ammon (16)] and mean Chao2 estimate of the overall generic richness with its 95% confidence interval (large circles with vertical bars) (table S1). PTB, Permian-Triassic boundary; 1, Kasimovian; 2, Gzhelian; 3, Asselian; 4, Sakmarian; 5, Artinskian; 6, Kungurian; 7, Roadian; 8, Wordian; 9, Capitanian; 10, Wuchiapingian; unlabeled successive intervals, Changhsingian, Griesbachian, Dienerian, Smithian; 15, Spathian; 16, Early Anisian; 17, Middle Anisian; 18, Late Anisian; 19, Ladinian; 20, Early Carnian; 21, Late Carnian; 22, Early Norian; 23, Middle Norian; 24, Late Norian; 25, Rhaetian. The end-Smithian ammonoid extinction event discussed in the text is not illustrated here due to its short time duration.

lack of statistical dependence between the numbers of sampled basins and singletons (Spearman $\rho = 0.364$, $P = 0.088$, not significant), this suggests that time bins with abundant singletons reflect intervals with high generic richness and turnover and are not spurious taxonomical, preservational, and/or sampling artifacts. For this reason, we only describe results based on S_{obs} .

The poly-cohort matrix (PCM) (Fig. 2) (17), numbers and rates of generic origination and extinction (Fig. 3 and tables S2 and S3), and phase diagram (fig. S4) show that ammonoid genera smoothly cross the Carboniferous-Permian boundary, in agreement with previous studies of the Carboniferous and Permian (8, 9). This stands in contrast with turnover at the family level (fig. S2). As shown before (4, 8), there is a slight increase in total diversity from the end-Carboniferous onward, culminating in the Middle Permian. Early-Middle Permian diversities are essentially governed by fluctuations in the Goniatitida (Fig. 1). During this time interval, the nonparallelism between the PCM's diagonal (the target assemblage axis) (fig. S5) and prenascence and survivorship contour lines (Fig. 2) emphasizes the relatively slow and uncoupled origination and extinction dynamics of Permian ammonoids (Fig. 3). During the Late Permian, temporal diversity patterns are marked by two well-known successive phases of severe extinctions (Capitanian and Changhsingian) (4, 9). This time interval of decreasing morphological diversity (8) corresponds to the progressive decline of Goniatitida and Prolecanitida and to the rise of Ceratitida (Ceratitina and Otoceratitina) (Fig. 1 and fig. S2). Prefiguring the post-PTB diversification dynamics, an increase in diversity observed during the Wuchiapingian stage, between the two major extinction events, resulted mainly from the rapid diversification of Otoceratitina, while Goniatitida experienced a marked diversity decline. This turnover is also well illustrated by the PCM graph and associated cluster analysis (Fig. 2), as well as by the phase diagram (fig. S4).

The Triassic part of the time series consists of four successive diversity oscillations of declining magnitude, probably primarily shaped by global climatic and oceanographic changes (1, 18)

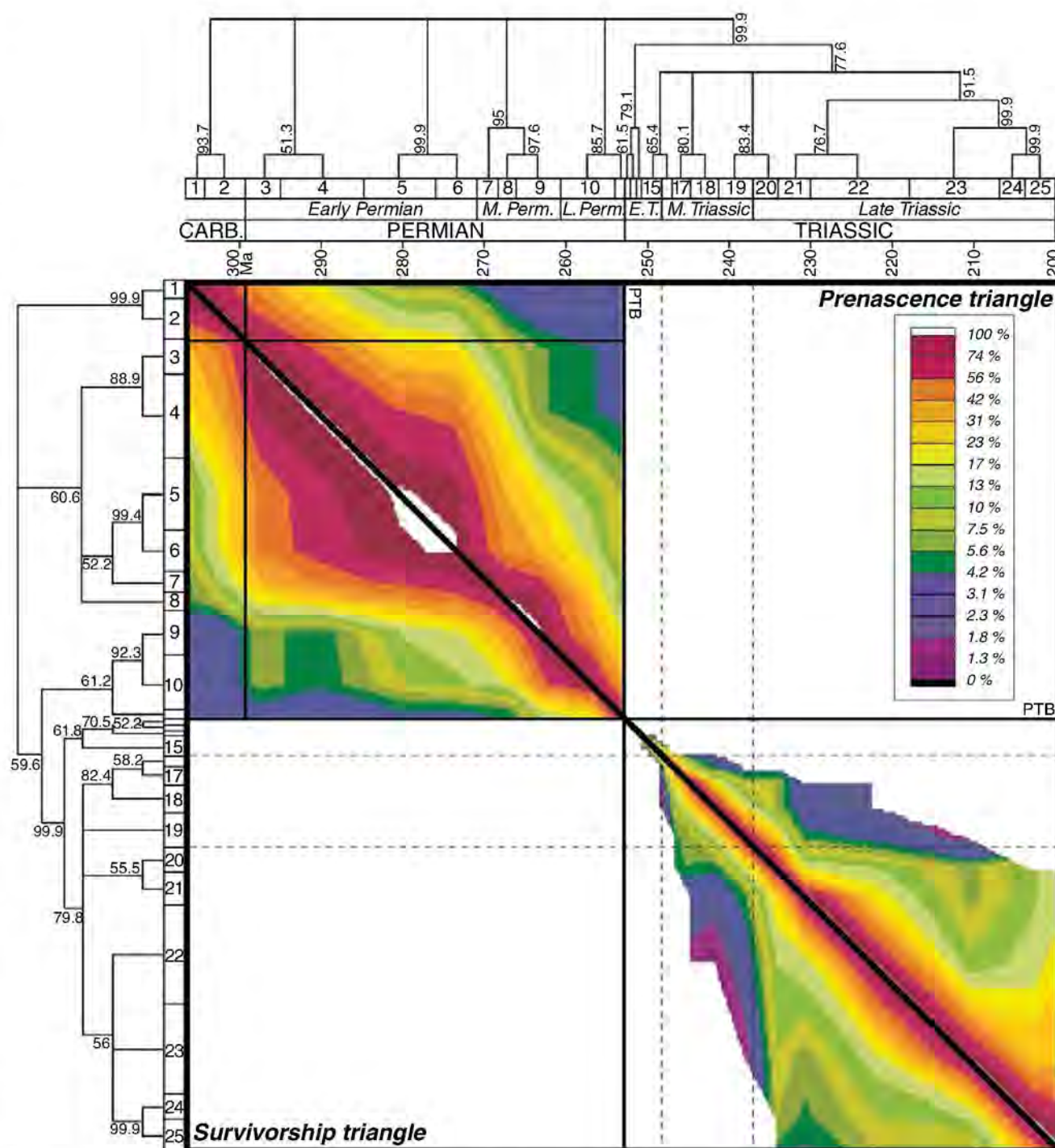


Fig. 2. Contour graph of the ammonoid poly-cohort matrix (PCM) (see fig. S5 for details). A poly-cohort is a set of taxa existing during a given time bin; the upper (prenascence) and lower (survivorship) PCM triangles record the origination and extinction dynamics of the target poly-cohort assemblages (PCM diagonal) as the percentage of taxa already or still existing before or after a time span δt , respectively. Associated trees are Neighbor-Joining majority rule consensus topologies (10,000 nonparametric bootstrap replicates; branch lengths not to scale) corresponding to the separate analysis of the origination and extinction dynamics (10); node percentages are estimated bootstrap supports. Same abbreviations as in Fig. 1.

(Figs. 1 and 3). In the first oscillation, during the Smithian—i.e., only 1 to 2 My after the PTB, based on the available radiometric ages and asso-

ciated uncertainties (10, 11)—ammonoid diversity reached values equal to, if not higher than, those for the Permian (~85 sampled genera) and

then were followed by still higher values during the Spathian (~110 genera). This late Early Triassic generic richness is unsurpassed during the Middle and Late Triassic, where diversity oscillated around an average value of ~70 sampled genera per time

bin, close to the Middle Permian maximum. This rapid recovery less than 2 My after a mass extinction is also seen for Early Jurassic ammonoids (19).

The Early Triassic rapid ammonoid diversification diverges from delayed recovery after the

PTB suggested for many benthic groups (e.g., 13). Apparently, recovery rates strongly varied across marine clades, and ammonoids boomed well before the oceanic realm returned to a long-term steady state. Our time-calibrated results indicate that there was a very rapid succession of new families and genera during the Early Triassic (Fig. 2 and figs. S2 and S4). Extreme contraction of survivorship and pre-nascence contour lines is diagnostic of high evolutionary rates, as echoed by the simultaneously high numbers and rates of Early Triassic originations and extinctions (Fig. 3). Ammonoid diversification during the Early Triassic produced more than 200 genera in less than ~5 My and was accompanied by a progressive change from cosmopolitan to latitudinally restricted distributions of genera (1). These changes coincide with the emergence of a clear latitudinal diversity gradient during most of the Smithian and Spathian, which suggests that the sea surface temperature gradient increased during the late Early Triassic (1, 2). This trend was not a gradual, continuous, and smooth one. For instance, ammonoid diversity severely dropped at the Smithian-Spathian boundary (1), coinciding with a major short-term perturbation of the global carbon cycle (11, 18, 20, 21). However, (i) ammonoids already reached a diversity level as high as, if not higher than, in the Permian during the Smithian (Fig. 1), and (ii) as for the end-Permian global event, the end-Smithian disturbances did not markedly delay the long-term Triassic ammonoid diversification.

How did these cephalopods flourish in the presumably unstable and harsh environmental conditions prevailing at that time? The same question applies to conodonts, whose Early Triassic diversity dynamics tend to parallel that of ammonoids (22). The mode(s) of life of ammonoids remains conjectural. These marine organisms may have a pseudoplanktonic juvenile stage (1, 23, 24). Ammonoids are morphologically and taxonomically so diverse that it is likely that they occupied a great variety of niches and exploited various food resources. Their high diversity and abundance suggest that diversified and abundant food resources were already available less than 2 My after the PTB. Consequently, even if Early Triassic trophic webs were possibly less complex than Permian and Middle-Late Triassic ones, they were far from devastated. At least some sizeable, while still unknown, primary production made it possible for these two clades to diversify profusely and rapidly despite short-term fluctuations of environmental conditions such as temperature, pH, and O₂ content of oceanic waters (e.g., 1, 18, 25).

The Early-Middle Triassic transition was again marked by a severe drop in ammonoid diversity. In this case, a fall in global sea level is implicated (26). Middle and Late Triassic generic and family richness remained lower than in the late Early Triassic, close to the Middle Permian maximum but with a few more families (~20 versus ~15 per time bin) (Fig. 1 and fig. S2); richness also appeared less variable, possibly because oceanic geochemical conditions stabilized during that time (11, 20, 21).

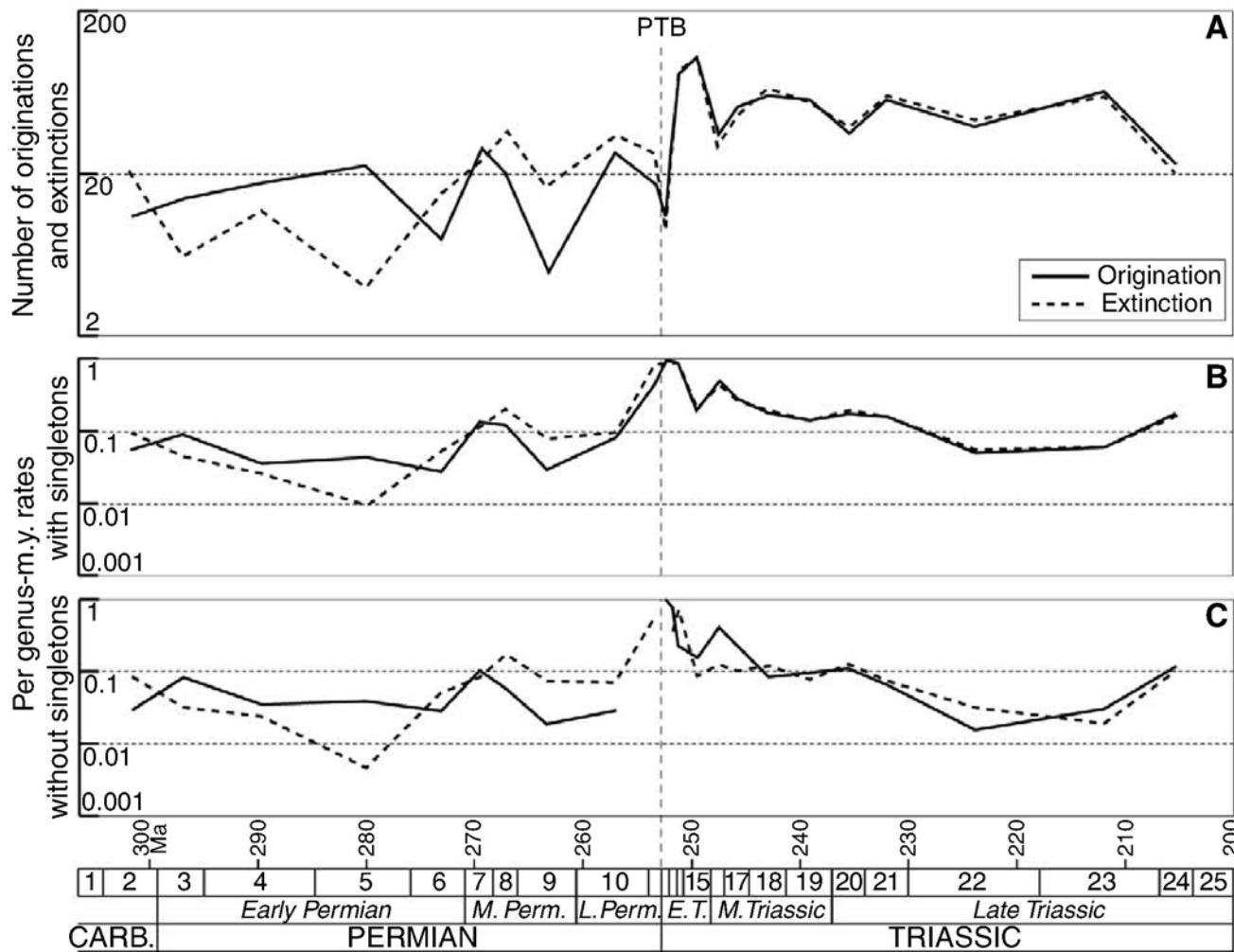


Fig. 3. Number of ammonoid genus origination and extinction events (including singletons, i.e., genera spanning only one time bin) (A), and Maurer's (28) per taxon-My origination and extinction rates with (B) and without (C) singletons, calculated for the 25 successive time bins (see table S2 for details). Same abbreviations as in Fig. 1.

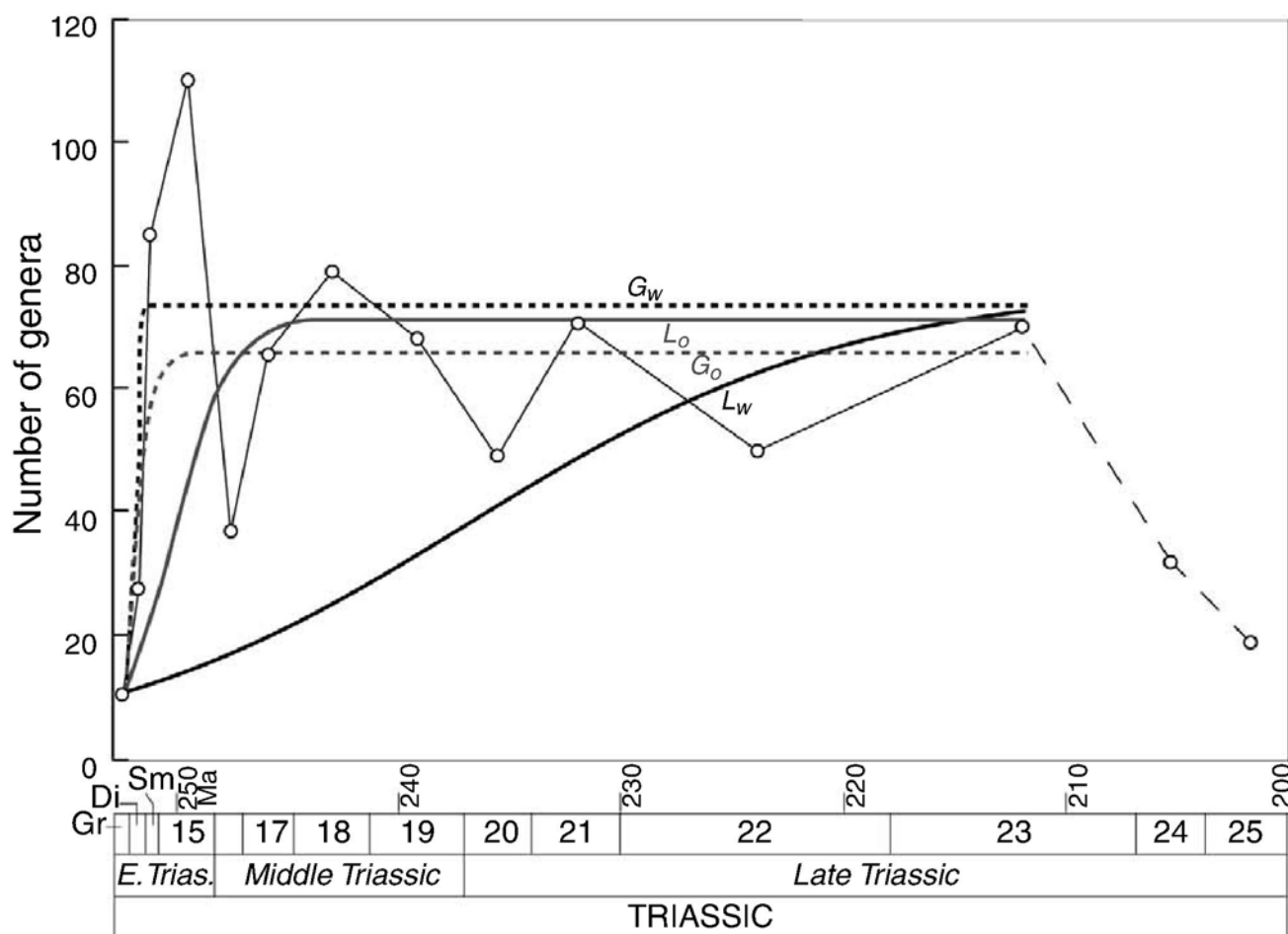


Fig. 4. Logistic (L) and hierarchical (G) models of diversification with (w) and without (o) singletons estimated for the Triassic ammonoid data set (tables S4 and S5). Same abbreviations as in Fig. 1. Gr, Griesbachian; Di, Dienerian; Sm, Smithian.

From the early Anisian onward, three successive diversity cycles are evident: Early Anisian–Early Carnian, Early Carnian–Early Norian, and Early Norian–Rhaetian (Figs. 2 and 3). The last cycle ends with a marked diversity decline through the Late Norian and Rhaetian, corresponding to the Triassic–Jurassic mass extinction (19).

Unlike in the Permian, where genera show uncorrelated to weakly correlated origination and extinction dynamics (Fig. 3 and table S2) [linear association between $\log(N_{Ori}^{t+1}/N_{Ori}^t)$ and $\log(N_{Ext}^{t+1}/N_{Ext}^t)$: $R^2 = 0.060$, $P = 0.56$ for Goniatic; $R^2 = 0.536$, $P = 0.025$ for Ammon], Triassic ammonoids show highly coupled dynamics ($R^2 = 0.978$, $P = 1.2 \times 10^{-9}$) because of the large average proportion of sampled singletons [71% versus 19% (Goniatic) to 53% (Ammon)] before the PTB]. We investigated the Triassic ammonoid diversity dynamics through diversification models directly derived from the empirical origination and extinction rates (Fig. 4). Based on the overall geometry of the sampled time series (excluding the Late Norian and Rhaetian time bins, which correspond to the end-Triassic mass extinction), we selected two diversity-dependent constrained diversification models: the (evolutionary-based) logistic (27) and (population dynamics-based) hierarchical (28) ones. These models basically differ in the way origination and extinction rates linearly (logistic) or exponentially (hierarchical) relate to taxonomic richness, ultimately leading to a sigmoidal-shaped curve of richness changing through time modeled as a two-parameter logistic function or a three-parameter Gompertz function, respectively. All computations were done based on Maurer's per-taxon per-My origination and extinction rates (28) computed with or without singletons (10). These rates share the same statistical behavior as Foote's estimated per-capita per-My rates (29), with which they highly correlate (table S3). We selected Maurer's rather than Foote's rates because the later cannot be calculated for three of the four Early Triassic time bins, that is, when ammonoids actually recovered (table S2).

Model comparison using evidence ratios calculated from corrected Akaike information criterion values favors the hierarchical diversification model over the logistic one (table S5). Indeed, even if both models converge toward the same steady-state richness values (~70 sampled genera) (Fig. 4), the logistic model clearly fails to capture the Early Triassic nondelayed recovery dynamics, contrary to the hierarchical one. In addition, the empirical (log) richness-rates relationships (table S4) illustrate a possible niche incumbency effect (30). This hypothesis, which predicts that richness and extinction rates are independent, allows the estimate of an average steady-state generic niche saturation level of ~85% under the hierarchical model, compatible with species niche saturation levels previously published for various clades of marine organisms (30).

Numerous Lazarus taxa among benthic and pelagic mollusks reappear during the Smithian (e.g., 6, 31). Coupled with the Triassic ammonoid

nondelayed diversity dynamics evidenced here, this suggests that complex trophic webs based on abundant and diversified primary producers were already functioning less than 2 My after the PTB and opens the possibility that heterotrophic taxa other than ammonoids also rapidly recovered. The end-Smithian global event, possibly linked to a late eruptive phase of the Siberian traps, initiated the conodont demise and corresponds to a major global change in the carbon cycle and climate (8, 18, 20, 21) but did not markedly delay the explosive recovery of Ceratitid ammonoids. This phased scenario for the Triassic biotic recovery accounts well for its generally accepted delayed character, which may reflect still inadequate sampling and time resolution and/or biased diversity estimates due to the lack of sampling standardization in the first million years after the PTB (32, 33). Recoveries obviously show environment- and clade-specific dynamics. Nevertheless, our results indicate that the time duration of the post-PTB recovery is likely overestimated, at least for some marine taxa.

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Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis

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Ecological restoration is widely used to reverse the environmental degradation caused by human activities. However, the effectiveness of restoration actions in increasing provision of both biodiversity and ecosystem services has not been evaluated systematically. A meta-analysis of 89 restoration assessments in a wide range of ecosystem types across the globe indicates that ecological restoration increased provision of biodiversity and ecosystem services by 44 and 25%, respectively. However, values of both remained lower in restored versus intact reference ecosystems. Increases in biodiversity and ecosystem service measures after restoration were positively correlated. Results indicate that restoration actions focused on enhancing biodiversity should support increased provision of ecosystem services, particularly in tropical terrestrial biomes.

Ecological restoration involves assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed, typically as a result of human activities (1). Restoration

actions are increasingly being implemented throughout the world (2), supported by global policy commitments such as the Convention on Biological Diversity [article 8(f), (3)]. A major

goal of ecological restoration is the reestablishment of the characteristics of an ecosystem, such as biodiversity and ecological function, that were prevalent before degradation (4). Increasing attention is being given to the value of ecosystems in providing ecosystem services [i.e., “the benefits people obtain from ecosystems” (5)]. There is a widespread assumption that ecological restoration will increase provision of ecosystem services (6, 7), but this has not yet been systematically tested.

Ecosystem services with high value for supporting human livelihoods include carbon storage, regulation of climate and water flow, provision of clean water, and maintenance of soil fertility (5, 8). A lack of scientific understanding of the factors influencing provision of ecosystem services and of their economic benefits limits their incorporation into land-use planning and decision-making (9). Many restoration actions are undertaken with the aim of increasing biodiversity (4). However, despite being the focus of major research attention, the relation between biodiversity and provision of ecosystem services remains uncertain (10). Restoration actions can provide insights into the dynamics and functioning of ecological systems as they constitute a form of experimental manipulation (4). Consequently, examination of the effects of restoration actions could provide insights into whether increases in biodiversity are likely to be associated with greater provision of ecosystem services.

Here, we describe a meta-analysis of 89 published scientific assessments of the outcomes of restoration actions undertaken in a variety of ecosystems from all continents except Antarctica. We used a standardized procedure to select restoration studies from scientific bibliographic databases on the basis of the comparators used and the measures made (11). In these studies, ecosystems had been degraded by a wide variety of processes (Table 1). Restoration actions generally included the removal or amelioration of the factor causing environmental degradation and/or the reestablishment of key ecosystem components to influence the rate and direction of recovery. The simplest approach was to cease the damaging activity—for example, the abandonment of agricultural land [“passive restoration” (12)]. Active restoration approaches are summarized in Table 1.

Assessment of the impacts of restoration actions typically involved field-based comparisons of different intervention treatments (11). Time scales of the restorations ranged from <5

to 300 years. To ensure suitable baselines (13) for examination of restoration success, we restricted our analysis to those studies that compared restored (Rest), reference (Ref), and degraded (Deg) ecosystems within the same assessment. We define reference ecosystems as those not subjected to the environmental degradation that the restoration was intended to redress. The degraded system therefore represented the starting point of the restoration and the reference system represented the desired end point.

From the 89 studies, we extracted 526 quantitative measures of variables relating to biodiversity and ecosystem services, which were incorporated into a database. The ecosystem services were classified according to the scheme developed by the Millennium Ecosystem Assessment (5), which distinguishes four categories: (i) supporting (e.g., nutrient cycling and primary production), (ii) provisioning (e.g., timber, fish, food crops), (iii) regulating (e.g., of climate, water supply, and soil characteristics), and (iv) cultural (e.g., aesthetic value). We examined only the first three services, because cultural services were not measured explicitly in any of the studies that we analyzed. Measures of biodiversity were related to the abundance, species richness, diversity, growth, or biomass of organisms present. We calculated response ratios (14) of the restored ecosystems compared with both the reference [$\ln(\text{Rest}/\text{Ref})$] and degraded [$\ln(\text{Rest}/\text{Deg})$] ecosystems for each measure of biodiversity and ecosystem services. The individual studies were classified into four broad biome types, according to whether they were aquatic or terrestrial and whether they were located in tropical or temperate regions (11) (table S1).

Using Wilcoxon signed rank tests, we examined whether the response ratios were different from zero to ascertain whether restoration affected biodiversity and the provision of ecosystem services. We also tested whether response ratios differed among ecosystem service categories and among biome types with the use of Kruskal-Wallis tests. Our results indicate that measures of supporting and regulating ecosystem services and biodiversity across the whole data set were higher in restored than in degraded systems (response ratio > 0, Fig. 1A) but lower than in reference systems (ratio < 0, Fig. 1B). Provisioning services showed no effect of restoration, but the sample size for this type of service was low. Our data indicate that supporting services, which provide the basis for provision of other services, were restored more effectively than other service types.

It is sometimes questioned whether restoration actions can be effective in enabling degraded ecosystems to acquire the characteristics of reference systems (13). Median values of response ratios showed that biodiversity and ecosystem services (all three types combined) in degraded systems were only 51 and 59%, respectively, of those in reference systems. Median response ratios of restored systems were sub-

stantially higher than those of degraded systems, with values of 144% for biodiversity and 125% for ecosystem services. However, the restored systems were not fully rehabilitated, as median response ratios for biodiversity and combined ecosystem services were 86 and 80%, respectively, of those in reference systems.

Biodiversity and provision of ecosystem services in restored ecosystems were more similar to degraded or reference ecosystems in aquatic than in terrestrial biomes and in temperate than in tropical biomes (Fig. 2). Response ratios were not significantly different from zero in tropical aquatic systems, probably because this biome had low sample size. The temperate aquatic biome showed significant effects of restoration only on biodiversity. When compared with degraded ecosystems, restoration was associated with the largest increases in ecosystem services and biodiversity in tropical terrestrial ecosystems (Fig. 2).

Theoretical and empirical work has identified a variety of linkages between changes in biodiversity and the way ecosystems function (15). We tested the hypothesis that a change in biodiversity is positively associated with altered provision of ecosystem services by correlating biodiversity and ecosystem service response ratios across studies. Treating each study as an

Table 1. Summary of the types of human activity that resulted in degraded ecosystems and the forms of restoration action undertaken in the 89 studies included in the meta-analysis.

Action	Number of studies
<i>Degrading action</i>	
Cessation of prescribed burning	3
Cultivation and cropping	13
Disturbance, excavation, or burial of substrate	15
Eutrophication	3
Hydrological disruption	21
Invasion by non-native species	4
Logging of trees	16
Over-grazing	5
Removal of carnivores or herbivores	3
Soil contamination	6
<i>Restoration action</i>	
Cessation of degrading action only (passive restoration)	13
Extirpation of damaging species (including non-natives)	8
Nutrient removal	3
Planting of forbs or grasses	12
Planting of trees	16
Reinstatement of burning	3
Reintroduction of herbivores or carnivores	3
Remodeling of topography	25
Soil amendments (to bind or dilute contaminants or restore fertility)	6

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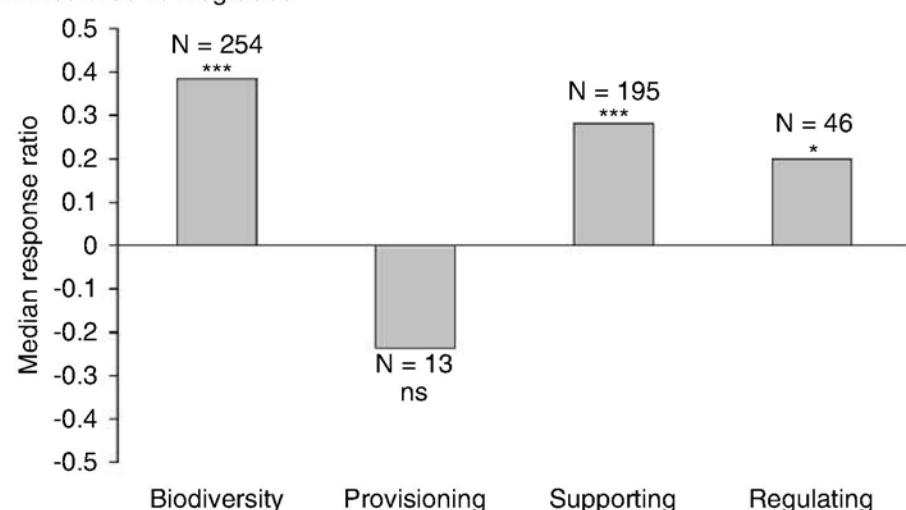
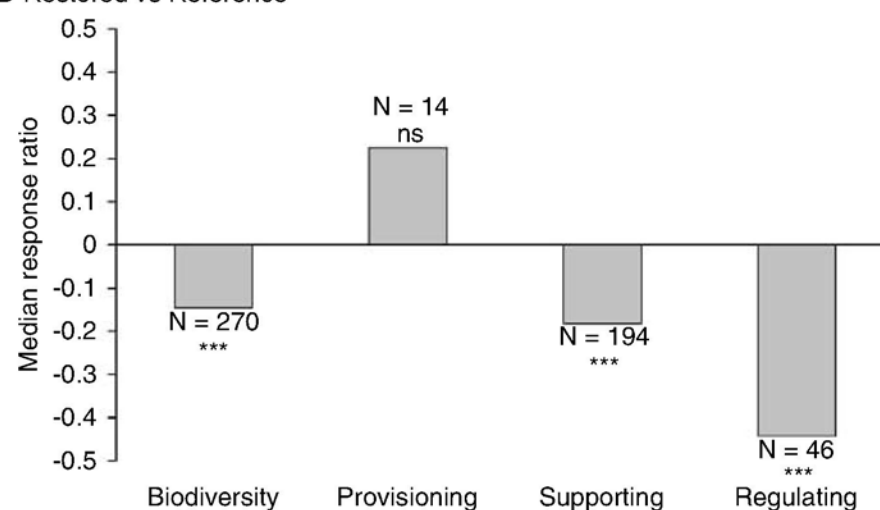
A Restored vs Degraded**B** Restored vs Reference

Fig. 1. Response ratios of biodiversity and ecosystem services in **(A)** restored compared with degraded ecosystems and **(B)** restored compared with reference ecosystems. All response ratios differed significantly from zero (Wilcoxon signed rank tests, *** $P < 0.001$, * $P < 0.05$), except those for provisioning services [not significant

(ns) $P > 0.05$]. Significant differences were found between the response ratios for biodiversity and the three ecosystem service categories with the use of Kruskal-Wallis tests [restored versus degraded: H (the K-W test statistic) = 11, N (sample size) = 508, $P < 0.05$; restored versus reference: $H = 15$, $N = 524$, $P < 0.01$].

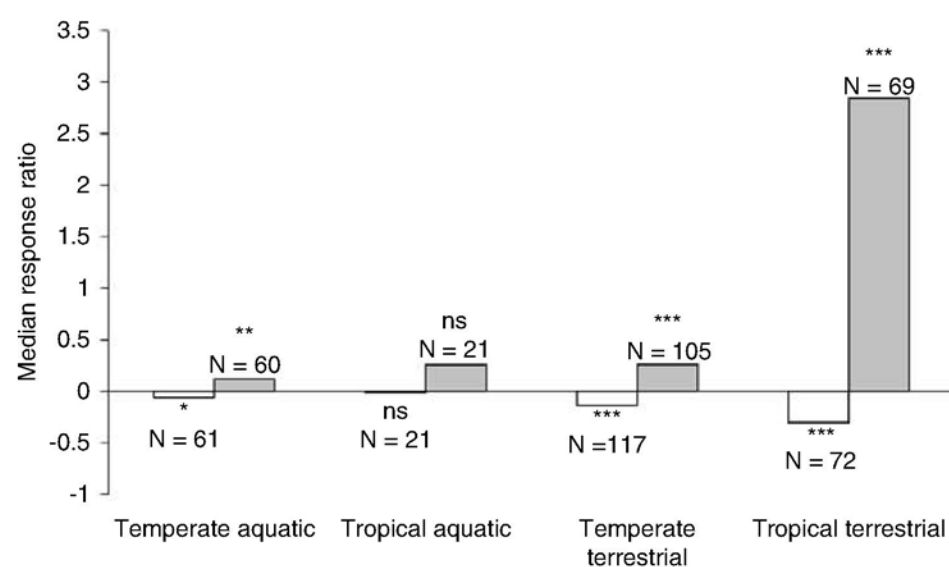
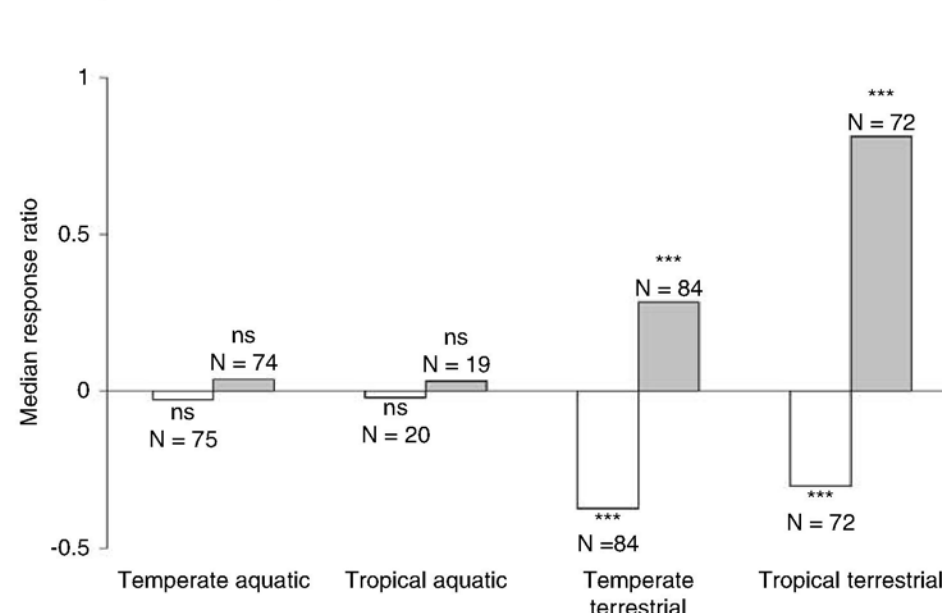
A Biodiversity responses**B** Ecosystem service responses

Fig. 2. Response ratios of **(A)** biodiversity and **(B)** amalgamated measures of ecosystem services in restored versus reference ecosystems and restored versus degraded ecosystems classified according to broad biome types. Except for biodiversity in the tropical aquatic biome and for ecosystem services in both temperate and tropical aquatic biomes, response ratios were significantly different from zero (Wilcoxon signed rank tests, *** $P < 0.001$,

** $P < 0.01$, * $P < 0.05$, ns $P > 0.05$) in each biome type. Kruskal Wallis tests showed significant differences among the biomes in the response ratios for biodiversity (restored versus reference: $H = 11$, $N = 271$, $P < 0.05$; restored versus degraded: $H = 61$, $N = 255$, $P < 0.001$) and ecosystem services (restored versus reference: $H = 25$, $N = 253$, $P < 0.001$; restored versus degraded: $H = 46$, $N = 251$, $P < 0.001$).

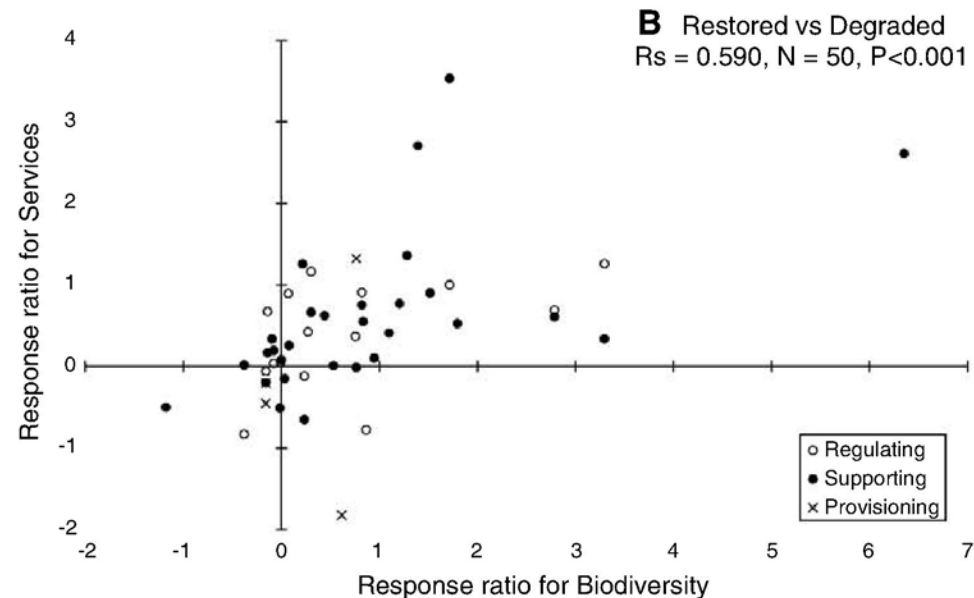
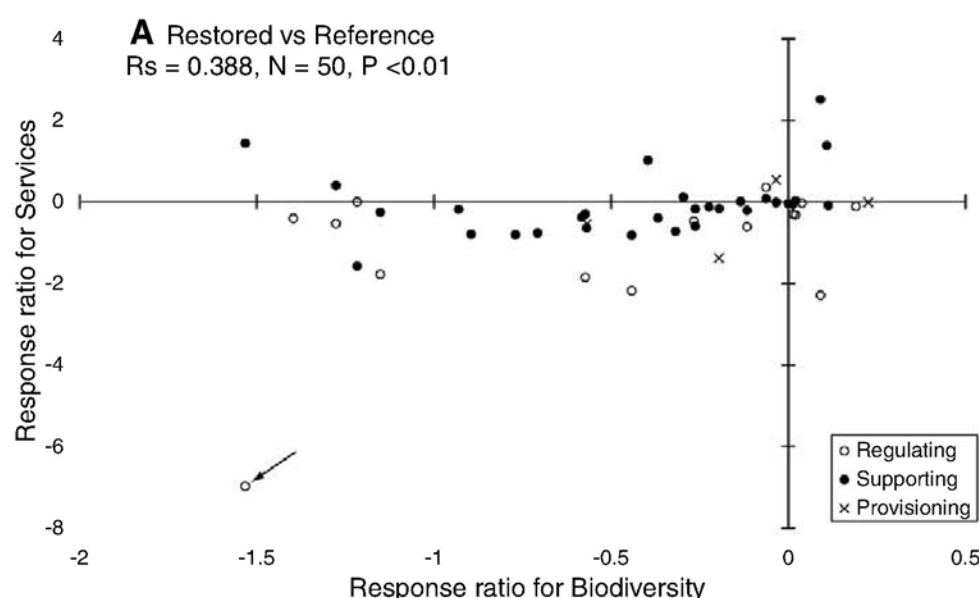


Fig. 3. Spearman rank (R_s) correlations between response ratios for biodiversity and for provision of ecosystem services in **(A)** restored versus reference ecosystems and **(B)** restored versus degraded ecosystems. The restored versus reference correlation remained significant after removing the outlier (indicated with an arrow) ($R_s = 0.353$, $P < 0.05$). Where multiple measures of biodiversity or

of a service were made in a study, pseudo-replication was avoided by averaging the response ratios to provide a single pair of values for biodiversity and each ecosystem service for analysis. To achieve a reasonable sample size, the different service types were combined, but here they are indicated by different symbols to illustrate the lack of systematic differences among them.

independent sample unit, Spearman rank correlation analysis showed that biodiversity and ecosystem service response ratios were positively correlated for both restored versus degraded and restored versus reference comparisons (Fig. 3). The relation was much stronger in the former comparison. This difference in the observed relations may be linked to an asymptotic relation between biodiversity and ecosystem function (15), whereby increasing biodiversity from low values has relatively strong impacts on individual ecosystem functions, but the relation plateaus at relatively high biodiversity values. Experimental investigations of the biodiversity-ecosystem function relation have generally been laboratory based or have employed small field plots (<100 m²), which arguably have little relevance to the larger scales (hectares to square kilometers) at which land management decisions are made (16). The current results support suggestions that when studies undertaken at a range of scales are combined, biodiversity is positively related to the ecological functions that underpin the provision of ecosystem services.

The relation between biodiversity and provision of ecosystem services is still poorly defined (10, 17). Preliminary mapping efforts at the global scale have shown that areas targeted for biodiversity conservation do not necessarily coincide with areas of relatively high provision of ecosystem services (10). However, conservation actions and investments typically occur at national, regional, and local scales. Our results suggest that, at such scales, ecological restoration is likely to lead to large increases in biodiversity and provision of ecosystem services, offering the potential of a win-win solution in terms of combining biodiversity conservation with socio-economic development objectives. Because ecological restoration can be effective in restoring natural capital, it should be implemented in areas that have undergone environmental degradation (18). The impacts of environmental degradation on human communities have been felt particularly heavily in tropical countries (19), where biodiversity loss and poverty are often associated (20). The meta-analysis showed the greatest impact of restoration in tropical terrestrial ecosystems, supporting the view that such management interventions could benefit human livelihoods in tropical regions (2).

Restoration actions cannot be implemented without incurring costs, and therefore, financial incentives will need to be provided for ecological restoration to be widely implemented (21). Potential approaches include improved markets and payment schemes for ecosystem services (22) and the Clean Development Mechanism developed under the Kyoto protocol. Cost-benefit analyses incorporating the values of biodiversity and associated ecosystem services and analysis of economic pathways are required to maximize return on investments in restoration (18). Restoration does not necessarily achieve the values of biodiversity or ecosystem services found in intact ecosystems, at least in the decadal time scales

adopted in the studies analyzed here, and this highlights the primary need to conserve wild nature and avoid environmental degradation wherever possible (23, 24). There is also a need to improve techniques for rehabilitating degraded ecosystems that will increase biodiversity and the provision of associated benefits to human society (12). Such techniques include improved monitoring of both biodiversity and ecosystem service outcomes of restoration actions.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 and S2

Table S1

References

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Unprecedented Restoration of a Native Oyster Metapopulation

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Native oyster species were once vital ecosystem engineers, but their populations have collapsed worldwide because of overfishing and habitat destruction. In 2004, we initiated a vast (35-hectare) field experiment by constructing native oyster reefs of three types (high-relief, low-relief, and unrestored) in nine protected sanctuaries throughout the Great Wicomico River in Virginia, United States. Upon sampling in 2007 and 2009, we found a thriving metapopulation comprising 185 million oysters of various age classes. Oyster density was fourfold greater on high-relief than on low-relief reefs, explaining the failure of past attempts. Juvenile recruitment and reef accretion correlated with oyster density, facilitating reef development and population persistence. This reestablished metapopulation is the largest of any native oyster worldwide and validates ecological restoration of native oyster species.

Along North American, European, and Australian coastlines, native oyster populations have been devastated to less than 10% of their historical abundance by overfishing and oyster reef destruction (1–3). These vital ecosystem engineers influence nutrient cycling, water filtration, habitat structure, biodiversity, and food web dynamics (3, 4). The widespread decline of these dominant suspension feeders was a leading cause of eutrophication in estuarine

ecosystems, owing to the shift from benthic to planktonic primary production and the accompanying hypoxia resulting from microbial decomposition (3). This phenomenon remains a major

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cause of ecosystem degradation in estuaries worldwide because of the largely failed efforts at oyster restoration (5). Consequently, non-native oyster species (for example, the Pacific oyster *Crassostrea gigas*) were introduced in many of these ecosystems to recover lost economic and ecological benefits (6), despite the unnatural alteration of the world's ecosystems (3, 5).

In the Chesapeake Bay, oyster landings of the native *C. virginica* peaked in the 1880s at 20 to 25 million bushels per year, whereas recent landings are less than 200,000 bushels (2). Concurrently, the natural populations were reduced to approximately 1% of their historical abundance (2, 3, 5), despite considerable expensive attempts to restore the populations. Introductions of *C. gigas* and other species were attempted through the 1900s, but these attempts failed because of biological and environmental impediments (7).

More recently, it was concluded that revival of the native oyster is unlikely and that the introduction of the non-native Asian oyster *C. ariakensis* merits consideration (8). This conclusion was based on the premise that restoration failed largely because of the inability of *C. virginica* to resist the challenge of two diseases: MSX, caused by the pathogen *Haplosporidium nelsoni*, and Dermo, caused by the pathogen *Perkinsus marinus*. However, various unfished populations have overcome disease pressure by being allowed to live in protected reefs that are conducive to growth, survival, and disease resistance (9–11). Moreover, the currently accepted strategy of attempting to restore the wild fishery and native populations in tandem allows for destructive harvest practices that devastate the structural integrity of reefs (12, 13) and inhibit recovery. Recently, however, scattered small assemblages of *C. virginica* have been observed on natural and alternative oyster reefs protected from exploitation in Delaware Bay (14), North Carolina sounds (12, 15), and the Chesapeake (16, 17), suggesting that restoration of the native *C. virginica* is feasible if novel methods are used.

We report the restoration of a native *C. virginica* metapopulation in the Great Wicomico River, a sub-estuary of the lower Chesapeake Bay that was selected for restoration in 2004 by the U.S. Army Corps of Engineers. Nine reef complexes covering 35.3 hectares (ha) were declared permanent sanctuaries, free from oyster fishing (fig. S1). Pre-restoration surveys demonstrated that there were, on average, less than two oysters per square meter throughout the nine reef complexes (18). The field experiment involved two restoration treatments [high-relief reef (HRR) and low-relief reef (LRR)] and a control treatment of unrestored bottom (UNB) spread over each of the reef complexes. In 2007, we sampled with patent tong and video surveys 85 1-m² plots, which were allocated randomly across the three treatments in the nine reef complexes (fig. S1) (18). We further sampled the reefs in March 2009 to verify the long-term persistence of the reefs.

In 2007, the metapopulation on the nine reefs consisted of an estimated 184.5 million oysters,

comprising 119.2 million adults of two age classes (2005 and 2006) and 65.3 million juveniles of the 2007 age class (fig. S2). These data indicate the protracted survival of settled individuals to adulthood and the recruitment of larvae to the reefs. This represents a 57-fold augmentation of the resident Great Wicomico River population (18), which greatly exceeds the previously unachieved restoration goal (a 10-fold increase of the 1994 baseline by 2010) of the Chesapeake Bay Program. Moreover, the reef complex has continued to develop and persists through April 2009, as evident in 2009 video and patent tong surveys (figs. S3 to S5).

The 185-million-strong population dwarfs all individual populations throughout Maryland's 111,600 ha of public oyster grounds in the upper Chesapeake Bay (19) and is nearly as large as the estimated 200 million oysters in all of Maryland's waters. Comparisons to native oyster populations in other parts of the world similarly demonstrate the unrivaled magnitude of the restoration. The largest documented populations of native oyster species comprise 24 million flat oysters *Ostrea anagasi* in Tasmania (20) and 100 million European flat oysters *O. edulis* in the Mediterranean (21). For all other native oyster species there are little data, but their populations are much smaller than the Great Wicomico River population (1, 3, 5, 6).

The major influence on oyster reef success was reef height, which drove abundance and density across the reef complexes (Fig. 1, figs. S3 to S5, and movies S1 to S6). Despite their much smaller area (12.1 ha), HRR segments harbored 67% or 123.8 million oysters (Figs. 1A and 2A, fig. S5, and movies S3 to S6), whereas the 23.2 ha of LRR contained 32% or 58.1 million (Figs. 1A and 2B, fig. S4, and movie S2), and 43.5 ha of UNB held only 1% or 2.6 million (Figs. 1A

and 2C, fig. S3, and movie S1). Irrespective of reef type, adults were twice as abundant as young juveniles (Fig. 1). Mean oyster density per square meter was fourfold higher on HRR (1026.7 ± 51.5 SE) than on LRR (250.4 ± 32.3 SE); UNB only had $6.0 (\pm 1.5$ SE) oysters/m² (Fig. 1B). The HRR density stands in sharp contrast to the typical average densities on Chesapeake Bay sanctuary reefs, which have 100 to 152 oysters/m². On harvested reefs in the Chesapeake Bay, oysters exist at much lower densities (2 to 11 oysters/m²); some harvested reefs harbor higher densities of up to 350 oysters/m², but these are rare.

The key feature mediating the abundant restored population was the vertical relief of the restored reefs, specifically the height above the river bottom (HRR, 25 to 45 cm, and LRR, 8 to 12 cm, before subsidence of 2 to 6 cm due to settling) of the oyster shell used to build the reefs. As the proportion of HRR increased on any particular reef, oyster density rose sharply from 200 oysters/m² when a reef was 10% HRR to over 1000 oysters/m² when a reef was 90% HRR (Fig. 3, $r^2 = 0.86$). For every 10% increase in the proportion of HRR, oyster density rose by 100 oysters/m² (Fig. 3). Similarly, oyster size (shell height) on HRR (47.3 mm ± 1.2 SE; fig. S2A) was 15% larger than that on LRR (41.0 mm ± 1.1 SE; fig. S2B). The mechanism mediating the superiority of HRR over LRR was most likely due to the optimal flow rates and corresponding healthier physiological condition of oysters on HRR, which maximizes growth and survival and minimizes disease influence and sedimentation (9, 12).

The sharply magnified oyster densities on HRR had two profound benefits for the long-term sustainability of the restored population (Fig. 4A) and the persistence of the associated reef matrix (Fig. 4B). First, there was a positive

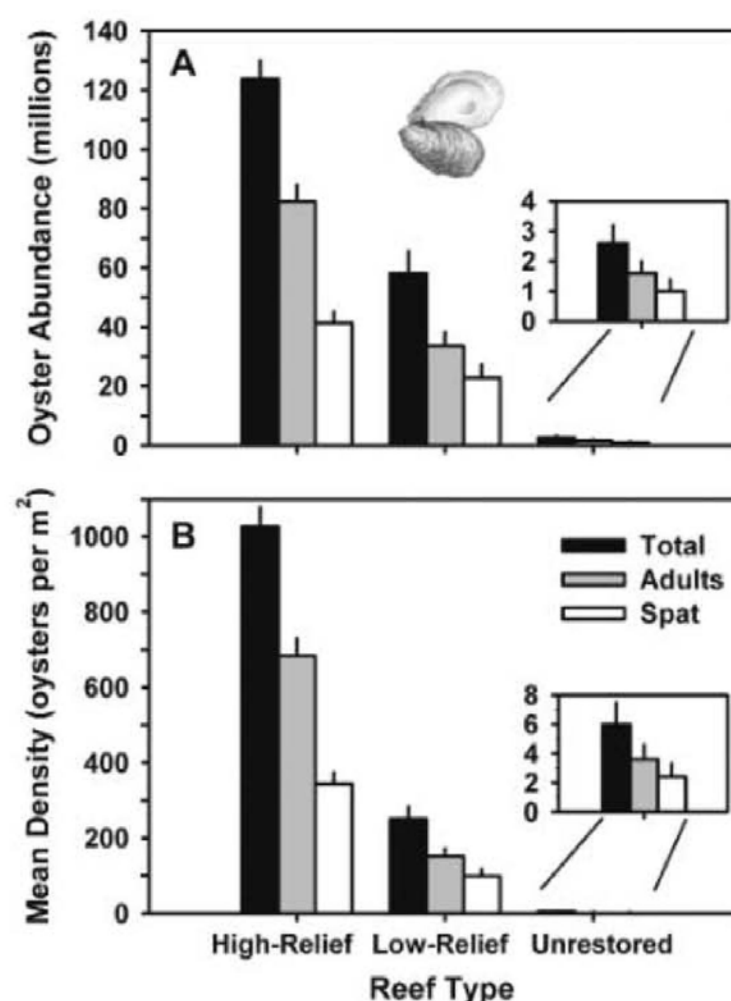


Fig. 1. (A) Oyster abundance and (B) density on each of the reef types across the nine-reef system. Values for UNB are magnified to demonstrate the similar pattern in adult and spat abundance as on HRR and LRR. Abundance over the system of nine reefs consisted of a total of 184.5 million oysters [95% confidence interval (CI): 165.0 to 204.0 million], 119.2 million adults (95% CI: 104.5 to 133.9 million) of the 2005 and 2006 year classes, and 65.3 million spat (95% CI: 59.7 to 77.2 million) of the newly recruited 2007 year class (fig. S1). Error bars represent 1 SE. $n = 22$ samples for HRR, 41 for LRR, and 22 for UNB.

Fig. 2. Underwater photographs representing average conditions for each of the reef treatments. (A) HRR, (B) LRR, (C) UNB.

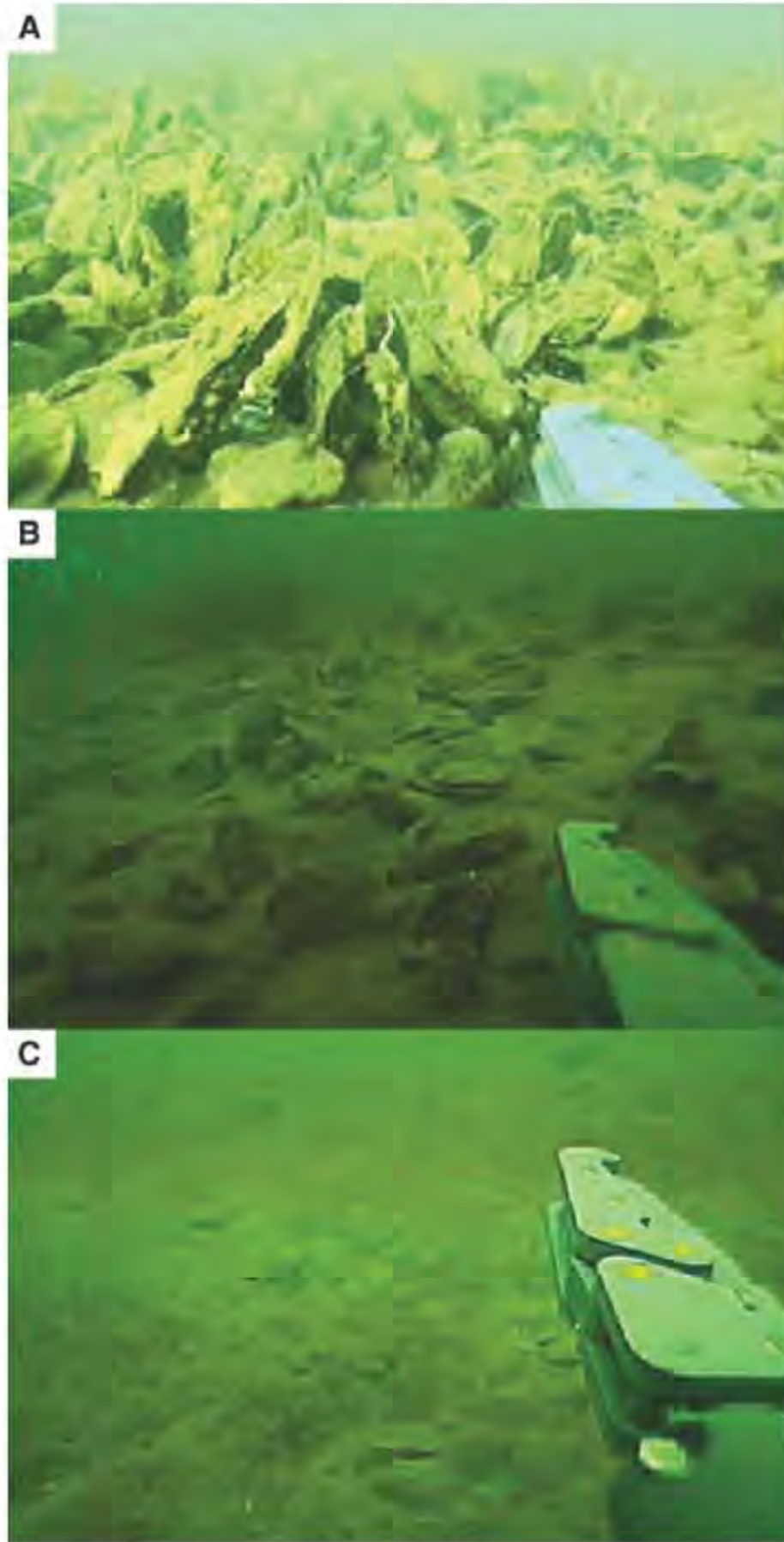
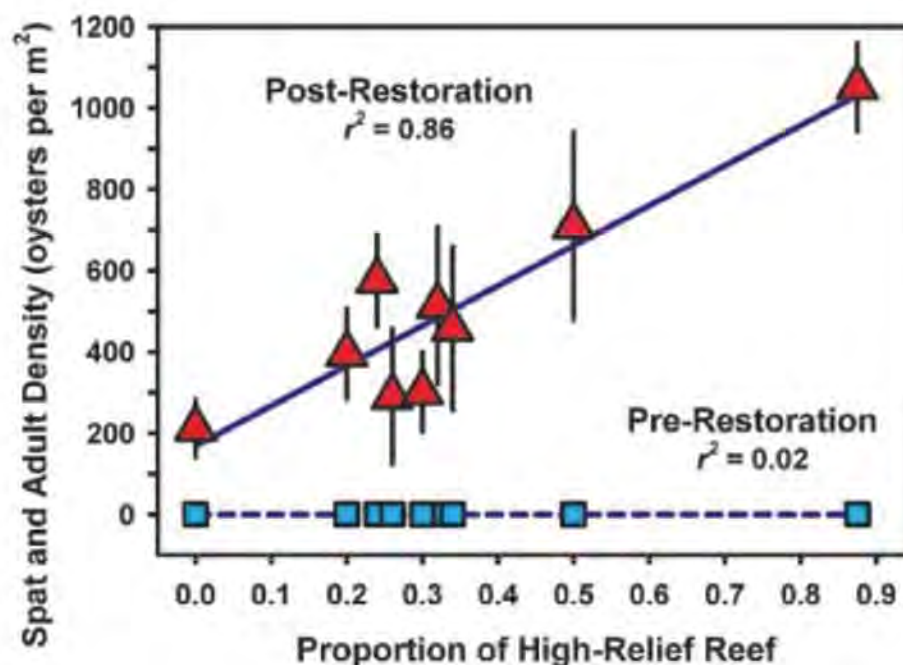


Fig. 3. Combined spat and adult oyster density as a function of the proportion of sampled HRR plots on each of the nine reefs [least-squares regression; spat and adult density = $165.5 + 992.8 \times (\text{proportion HRR})$; $r^2 = 0.86$, $n = 9$ reef systems].

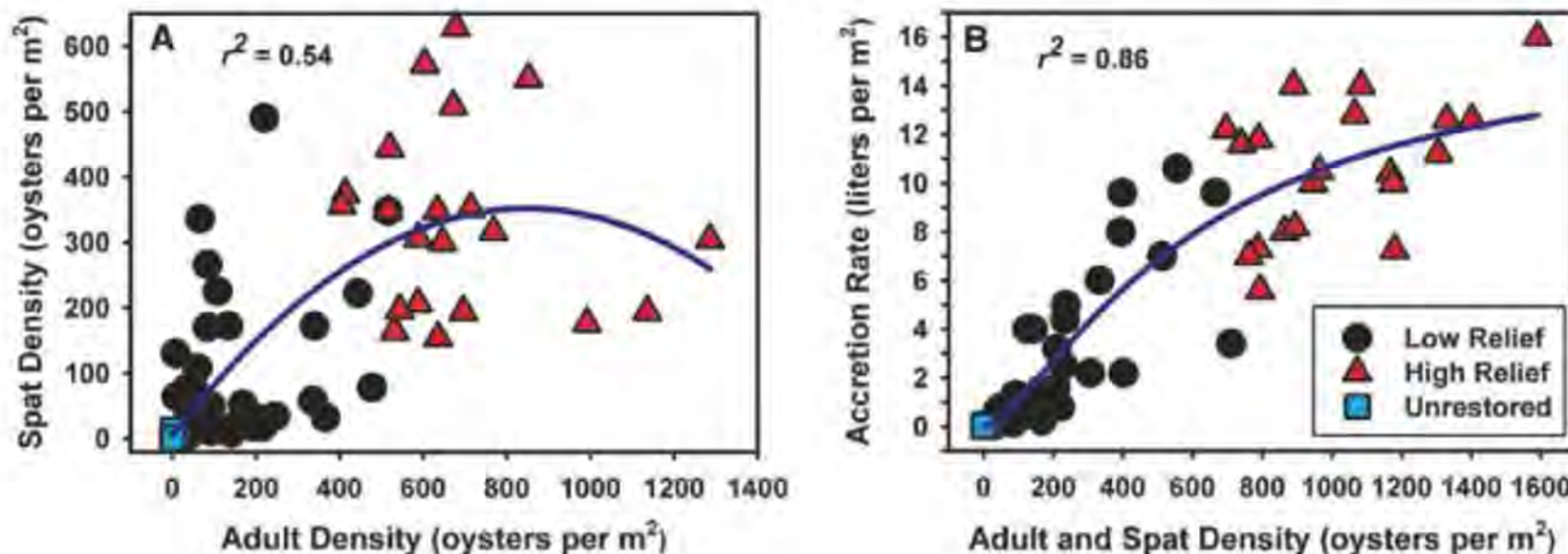


feedback between adult density and subsequent juvenile recruitment, such that spat density was a positive parabolic function of adult density, with a peak at an adult density of 850 oysters/m², after which juvenile recruitment declined (Fig. 4A). Variance in juvenile recruitment also differed by reef type (Fig. 4A) and was distinctly lower on HRR (coefficient of variation = 43%) than on LRR (129%). Thus, recruitment was not only much greater on HRR, but it was also more consistent than the variable and lower recruitment on LRR (Fig. 4A).

Oyster reefs require an accumulation of accreting shell (that is, the conglomeration of shell from living and dead oysters) that develops vertically with a complex architecture and serves as the base for the extant population, spat settlement, and reef persistence. The accretion rate of shell material on restored reefs was a sigmoid function of total oyster density and differed substantially by reef type: 6 to 16 l m⁻² on HRR and <4 l m⁻² on LRR (Fig. 4B). Historically, accretion rates exceeding 5 l m⁻² characterized successful native oyster reefs (22). The vertical growth and cohesiveness of HRRs indicate that they are coalescing into the historic, natural oyster reef architecture typical of pre-exploitation reefs (22), as evident in the photographs and video clips from 2007 and 2009 (Fig. 1, fig. S5, and movies S3 to S6). These results suggest that oyster reefs exist in two alternative states (23), one a heavily sedimented degraded state and the other a vertically accreting, elevated reef configuration composed of abundant oysters, which provides a positive feedback to reef integrity.

It has been suggested recently that native oyster restoration cannot succeed, because restored reefs do not accrete reef material at sufficient rates to compensate for losses due to shell degradation and sedimentation (24, 25). This conclusion is based on data from restored reefs that are characterized by poor habitat quality (for example, low reef height), low recruitment, low standing stock, and ongoing exploitation, which destroys the reef architecture and removes large adults from the population (13). Such reefs are comparable to the poorly performing LRR in the Great Wicomico River. In contrast, HRRs are accreting shell at rates significantly faster than 5.0 l m⁻² year⁻¹, indicating that HRR has developed into a robust, permanent reef structure, whereas much of the LRR is not likely to persist for more than a few years. The HRRs exhibit both vertical and cohesive growth, in contrast to the pattern of reef degradation that was typically observed on previous native oyster restoration projects (26). Our patent tong samples and underwater observations with a remotely operated vehicle in March 2009 indicate that recruitment of the 2008 year class was very successful and that the reefs are continuing to develop and grow, attesting to the expansion and persistence of the reef matrix. The HRR system has persisted and, more importantly, thrived for nearly 5 years, well past the typical longevity of

Fig. 4. (A) Spat density as a function of adult density and **(B)** accretion rate as a function of adult and spat density on each of the 85 1-m² plots sampled across the nine reefs. [Nonlinear least-squares regression: (A) spat density = $4.672 + 0.818 \times (\text{adult density}) - 0.001 \times (\text{adult density})^2$; $r^2 = 0.54$, $n = 85$ samples; (B) accretion rate = $-0.302 + 16.860 \times (1 - e^{-0.001 \times \text{adult and spat density}})$; $r^2 = 0.86$, $n = 85$ samples].



failed oyster reefs (26). The HRRs are gaining shell material and establishing oyster densities at rates previously unrecorded on native oyster restoration projects.

The native oyster metapopulation on the restored reef system in the Great Wicomico River greatly exceeds the recently proposed criteria for sustainability (15): (i) It comprises multiple year classes at high abundance, which buffers year-to-year variation in spat settlement; (ii) it is composed of young and old adults that have survived disease challenge; (iii) the reefs are accreting (that is, growing) at a rate that will provide settlement habitat for future generations; and (iv) it receives sufficient spat settlement and recruitment to sustain the population over the long term.

The recent recovery of a native *C. virginica* metapopulation in the Great Wicomico River of the Chesapeake Bay, as well as limited successes in other North American estuaries (14–17), highlight the critical importance of two common features of successful reefs: protection from fishing and high vertical relief (9, 10, 12, 13). Past oyster restoration efforts operated under the mistaken premise that fishery and ecological restoration could be accomplished simultaneously (7, 8). This approach failed to stem the decline in oyster stocks and led to the widespread use of more efficient fishery methods such as power dredging, which is the most destructive technique of harvesting oysters (13, 26). This strategy promoted partial fishery recovery via put-and-take fisheries at the expense of ecological restoration, and consequently perpetuated the precipitous decline of oyster populations in the Chesapeake Bay as well as along the Atlantic and Gulf of Mexico coasts of North America (1–3, 5).

The Great Wicomico River restoration project deviated markedly from prior restoration attempts in the Chesapeake Bay by building oyster reefs of high vertical relief at a broad spatial scale in large sanctuaries that are protected from fishery exploitation and in locations characterized by high recruitment (19, 27). Typical restored sanctuaries before this project amounted to less than 1% of an estuary's historical oyster reef footprint. The Great Wicomico River reef

network encompasses approximately 40% of the original oyster reef footprint (28) within a hydrodynamically restricted system (27). This metapopulation connectivity promotes persistence of individual populations in the network and larval subsidies from protected source reefs to fished reefs (29) with the attendant ecological and economic benefits (4). Designation of the reefs as sanctuaries protects the reefs both from exploitation of the spawning stock and physical destruction of the critically important vertical structure. Significant vertical relief and reef persistence were accomplished by building a substantial portion of the reef system as high as 45 cm (HRR) in contrast to the 8- to 12-cm LRR, which typically does not promote reef persistence for more than 3 to 5 y (26). LRRs have been the construction method of choice by fishery management agencies in the Chesapeake and several other estuaries. The ephemeral nature of LRRs has proven to be one of the main impediments to the recovery of native oyster habitat wherever they are used.

The vertical growth and cohesiveness of HRRs indicate that they are coalescing into the historic natural architecture typical of pre-exploitation oyster reefs (30), as evident in our photographs and video clips (Fig. 1, fig. S5, and movies S3 to S6). Winslow (30), during his historic survey of oyster reefs, documented perhaps the last unexploited reefs in the Chesapeake Bay. These reefs consisted of “long, narrow oysters . . . no single oysters of any [age] class, but all grew in clusters of 3 to 15. The shells were clean and white, free from mud and sand. The mature oysters were covered and the interstices between them filled with younger oysters.” Moreover, he noted that it was very difficult to sample these reefs due to their cohesive nature, which we also experienced when attempting to sample HRRs during our 2009 survey.

Although disease will kill some oysters in the Great Wicomico River, the recent development of disease tolerance in oysters on sanctuary reefs of the lower Chesapeake Bay (11) bodes well for the long-term persistence of this metapopulation and its attendant ecosystem benefits (4). Similar approaches with other natural (14) and artificial (31) reefs could lead to recovery of the native

oyster throughout North America, as well as in other ecosystems worldwide, where native oysters have been functionally extirpated (3, 5).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1176516/DC1
Materials and Methods
Figs. S1 to S5
References
Movies S1 to S6

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Functional Characterization of the Antibiotic Resistance Reservoir in the Human Microflora

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To understand the process by which antibiotic resistance genes are acquired by human pathogens, we functionally characterized the resistance reservoir in the microbial flora of healthy individuals. Most of the resistance genes we identified using culture-independent sampling have not been previously identified and are evolutionarily distant from known resistance genes. By contrast, nearly half of the resistance genes we identified in cultured aerobic gut isolates (a small subset of the gut microbiome) are identical to resistance genes harbored by major pathogens. The immense diversity of resistance genes in the human microbiome could contribute to future emergence of antibiotic resistance in human pathogens.

Multiple antibiotic resistance in human pathogens has increased over the past decades and challenged our ability to treat bacterial infections (1, 2). For example, methicillin-resistant *Staphylococcus aureus* (MRSA) caused 18,964 mortalities in the United States in 2006 (3). The comparison with 14,627 AIDS-related mortalities that occurred in the same year (4) highlights the public health importance of just one multiresistant bacterial pathogen in an industrialized nation. Whole-genome sequencing of bacteria has revealed that many of the resistance genes harbored by these strains have not evolved within the sequenced strain but were acquired by lateral gene transfer events (5). Antibiotic resistance determinants encoded on mobilizable elements move between diverse bacteria to disseminate resistance genes into a variety of interacting microbial communities (6, 7). Consequently, there is an increasing interest in elucidating reservoirs of mobile antibiotic resistance genes that may be accessible to clinically relevant pathogens (8–10).

The human microbiome substantially impacts human health and plays beneficial roles in dietary processing and prevention of pathogen intrusion (11–15). The widespread use of antibiotics in human medicine and agriculture has likely induced substantial responsive changes in this community.

Phylogenetic analysis of human and mice gut microbiomes reveal substantial transient increases in the Proteobacteria at the expense of Firmicutes and Bacteroidetes during antibiotic treatment (16, 17). Although the phylogenetic distribution of the human gut microbiome was found to largely reestablish after ciprofloxacin treatment (18), it has likely been enriched in resistance to this antibiotic. Indeed, persistent clarithromycin resistance in commensal enterococci has been observed years after cessation of antibiotic therapy (19), and resistance toward amoxicillin has been observed in cultured oral bacterial isolates from children not exposed to antibiotic therapy (20). Previous work has also shown that the abundance of *ermB* and *tetQ*, encoding resistance to erythromycin and tetracycline, respectively, has increased in frequency in cultured human *Bacteroides* isolates during the past three decades and that *ermB* has been exchanged between distantly related cultured commensal gut bacteria (21). These findings

suggest that the human microflora could constitute a substantial reservoir of antibiotic resistance genes accessible to pathogens (22, 23). We used functional selections coupled with metagenomic analysis to characterize the antibiotic resistance genes in the oral and gut microflora of healthy individuals.

We isolated DNA directly from saliva and fecal samples from two unrelated healthy humans who had not been treated with antibiotics for at least 1 year (24). One- to three-kilobase fragments of the purified metagenomic DNA were cloned into an expression vector and transformed into an *Escherichia coli* host strain, resulting in libraries of a total size of 9.3×10^9 base pairs. Antibiotic-resistant clones from each library were selected by plating on Luria broth agar containing one of 13 antibiotics belonging to the classes amino acid derivatives, aminoglycosides, amphenicols, beta-lactams, and tetracyclines, at concentrations where the wild-type host strain is susceptible (table S1) (24). Inserts conferring resistance to all 13 antibiotics tested were sequenced and annotated, enabling the discovery of 95 unique inserts containing functional antibiotic resistance genes (table S3) (24).

On average, the sequence similarity of the resistance genes we obtained and the closest related gene in GenBank is 69.5% at the nucleotide level (Fig. 1A) and 65.3% at the amino acid level (Fig. 1B) (24). A minority (22%) had high homology (>90% amino acid identity) to previously known genes, including identical matches to the *tet(Q)*-3 gene previously identified in cultured *Bacteroides* isolates from the human colon (21) and the CTX-M-15 enzyme, which over the past decade has become one of the most prevalent extended-spectrum beta-lactamases (Table 1) (25). Most of the closely related homologs are derived from commensals including nonpathogenic species such as

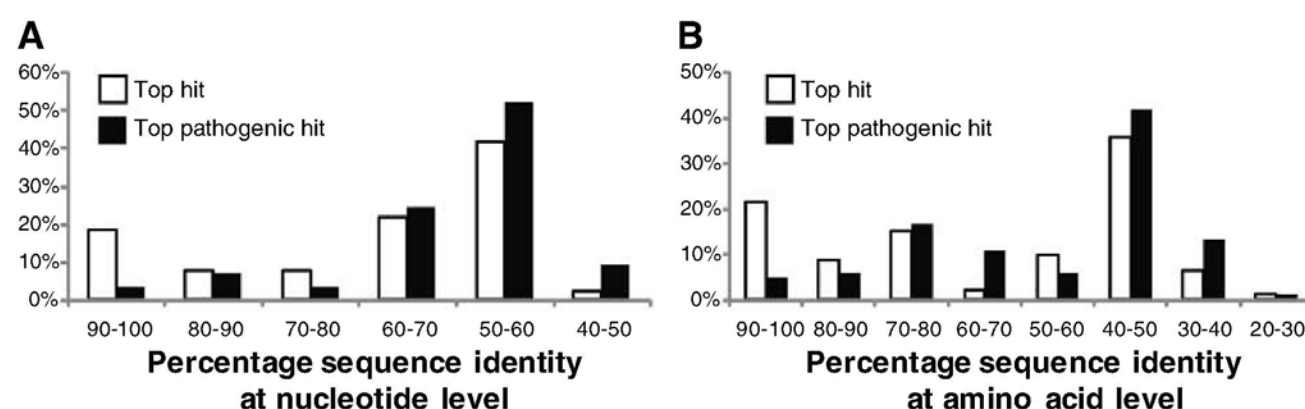


Fig. 1. Distributions of (A) nucleotide identities and (B) amino acid identities for 93 resistance genes identified from DNA extracted directly from saliva and fecal samples to the most similar resistance gene from any organism (white bars) as well as the most similar resistance gene harbored by a pathogenic isolate (black bars) in GenBank (table S3) (24). Two resistance genes with no significant similarity to sequences in GenBank are not shown.

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Bifidobacterium longum (26), as well as commensals with the capacity to become opportunistic pathogens such as *Bacteroides fragilis* and *Bacteroides uniformis* (table S3) (21). Interestingly, we also identified genes that encode proteins that are 100% identical to hypothetical proteins of unverified function in GenBank, for example, BACUNI_02013 from *Bacteroides uniformis* ATCC 8492, which we show encodes resistance to broad-spectrum beta-lactams such as amoxicillin and carbenicillin, and the third-generation oxyimino-cephalosporin cefdinir (table S3) (27). This highlights the utility of a functional selection approach to improve annotation of genomic and metagenomic sequencing data from the human microbiome project (28).

Most of the antibiotic resistance genes harbored by the human microflora were distantly related (60.7% at the nucleotide level and 54.9% at the amino acid level) to antibiotic resistance genes so far detected in pathogenic isolates (Fig. 1 and table S3) (24). In total, we identified 78 unique inserts with genes with low homology (<90% amino acid identity) to proteins in GenBank, encoding resistance to the 13 antibiotics profiled (tables S1 and S3). This may imply that the resistance genes of the human microbiome are inaccessible or infrequently exchanged with human pathogens; however, all the resistance genes we

identified in this study were functional in *E. coli*, which suggests that if a barrier to gene transfer exists between the constituents of the human microbiome and pathogens, it must stem from processes other than functional compatibility.

Phylogenetic analysis of the inserts using PhyloPythia (29) indicates that they predominantly originate from the phyla Bacteroidetes and Firmicutes, which dominate the gut flora (14). However, the majority of the genes we discovered have low sequence identity to resistance genes previously identified in pathogens from these phyla (e.g., *Staphylococcus aureus* and *Streptococcus pneumoniae*), as well as from the numerous pathogens that are readily culturable facultative anaerobic bacteria from the phylum Proteobacteria. Although commensal Proteobacteria constitute less than 1% of the human gut microflora (14), they increase in abundance during antibiotic treatment at the expense of the normally abundant Bacteroidetes and Firmicutes (16, 17). As a consequence of their normal low abundance in healthy individuals, they are not well represented in unbiased metagenomic libraries.

We isolated 572 bacterial strains on rich media under aerobic conditions from fecal samples from two healthy individuals (24). Phylogenetic profiling revealed that they belonged primarily to

Proteobacteria, with a few Firmicutes and Actinobacteria (fig. S1). The isolates from individuals 1 and 2 were on average resistant to 9 and 5 out of 13 antibiotics, respectively (Fig. 2, C and D). Chloramphenicol and minocycline were the only antibiotics tested that were able to prevent the growth of more than 99% of the isolates (Fig. 2, A and B, and figs. S2 and S3).

Functional selections identified 115 unique inserts encoding transferable antibiotic resistance genes from the cultured aerobic gut microbiome isolates (Fig. 3 and table S4) (24). We found that 95% of these genes are over 90% identical at the nucleotide level to resistance genes in pathogenic isolates, and almost half of these genes were 100% identical (Fig. 3A), indicating an evolutionarily close relationship to the resistance genes harbored by clinical pathogens.

The group of resistance genes identical to those in pathogens belong to one class of tetracycline efflux pumps (*TetA*), two classes of aminoglycoside-modifying enzymes [*AAC(3)-II* and *AAC(6)-Ib*], and three classes of beta-lactam-inactivating enzymes (*TEM*, *AmpC*, and *CTX-M*) (Fig. 4). We identified a *TEM-1* gene variant (Fig. 4 and Table 1) in cultured isolates from one gut microbiome on every sampling time (24) that has recently been reported in pathogenic strains of

Table 1. Unique beta-lactamase genes identified from gut and oral microbiomes from healthy humans. Gene ID refers to unique identifier in tables S3 and S4, and enzyme names use established nomenclature (24, 32). Percentage

amino acid identity to the closest related gene in GenBank is calculated using the global alignment program clustalW (24, 33). NCBI, National Center for Biotechnology Information.

Beta-lactamase family	Enzyme name	Gene ID	GenBank ID	Source	Amino acid identity to NCBI (%)
AmpC	AmpC-EcolK12	AX_iG2_08	GQ343010	Aerobic gut isolate	100.0
	AmpC-EC6	PE_iG1_02	GQ343155	Aerobic gut isolate	100.0
	AmpC-EC31	PE_iG2_05	GQ343162	Aerobic gut isolate	100.0
	AmpC-HG1	AX_iG2_21	GQ343018	Aerobic gut isolate	99.7
	AmpC-HG2	CA_iG2_12	GQ343059	Aerobic gut isolate	99.5
	AmpC-HG3	CF_iG2_01	GQ343071	Aerobic gut isolate	90.0
	AmpC-HG4	CF_iG2_06	GQ343073	Aerobic gut isolate	97.4
	AmpC-HG5	CA_iG1_06	GQ343055	Aerobic gut isolate	99.2
TEM	TEM-1b	AX_iG1_01	GQ343004	Aerobic gut isolate	100.0
	TEM-168	PE_iG2_13	GQ343167	Aerobic gut isolate	99.7
	TEM-169	PI_iG2_05	GQ343173	Aerobic gut isolate	99.1
CTX-M	CTX-M-15	AX_iG1_04	GQ343005	Aerobic gut isolate and metagenomic gut sample	100.0
	CblA-1	AX_iG2_02	GQ343019	Aerobic gut isolate and metagenomic gut sample	100.0
CblA	CblA-2	AX_mG2_03	GQ342999	Metagenomic gut sample	99.7
	CblA-3	PE_mG2_02	GQ343154	Metagenomic gut sample	99.0
CfxA	CfxA6	AX_mG2_01	GQ342996	Metagenomic gut sample	87.2
HGA	HGA-1	CA_mG1_02	GQ343038	Metagenomic gut sample	61.4
HGB	HGB-1	AX_mG2_05	GQ343000	Metagenomic gut sample	58.5
HOA	HOA-1	AX_mO1_01	GQ343035	Metagenomic saliva sample	49.5
HGC	HGC-1	CA_mG1_01	GQ343037	Metagenomic gut sample	48.1
	HGC-2	CA_mG1_04	GQ343039	Metagenomic gut sample	51.0
HGD	HGD-1	CA_mG2_04	GQ343044	Metagenomic gut sample	52.9
HGE	HGE-1	AX_mG2_11	GQ343003	Metagenomic gut sample	37.1
HGF	HGF-1	AX_mG2_09	GQ343002	Metagenomic gut sample	43.3
HGG	HGG-1	PE_mG1_01	GQ343153	Metagenomic gut sample	38.8
HGH	HGH-1	PI_mG1_01	GQ343170	Metagenomic gut sample	34.5
HGI	HGI-1	CA_mG2_07	GQ343045	Metagenomic gut sample	42.6

E. coli, *Salmonella enterica*, *Klebsiella pneumoniae*, *Haemophilus parainfluenzae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis* isolated around the globe (table S2). Nearly 80% of the depositions of this *TEM-1* variant to GenBank have occurred between 2007 and 2008, which seems to indicate a relationship between the emergence of this resistance gene variant in the clinic and its occurrence in healthy humans.

The *AmpC* and *CTX-M* family of enzymes are extended-spectrum beta-lactamases that hydrolyze a wider variety of later generation beta-lactams (Table 1) (30). We identified the *CTX-M-15* beta-lactamase (Fig. 4 and Table 1) in libraries from cultured gut microbiome isolates across multiple sampling days, as well as in our metagenomic libraries from the same microbiome.

Global sequence alignments of each of the 27 unique beta-lactamase sequences from our study identified 15 distinct sequence groups (Fig. 4). Of these groups, 5 were previously characterized (*CblA*, *CfxA*, *CTX-M*, *TEM*, and *AmpC*) (31), whereas 10 constitute previously unknown beta-lactamase sequence families because they are between only 35% and 61% identical at the amino acid level to any gene products in GenBank (Fig. 4). Interestingly, the known beta-lactamase genes we identified were from the cultured microbiome isolates, but the 10 previously unidentified gene families were identified solely by our culture-independent characterization (Fig. 4). In general, we found that the metagenomically derived resistance genes in our study were more distantly related to previously identified genes than those derived from aerobic gut isolates (Figs. 1 and 3 and figs. S5 and S6).

Of our 210 unique microbiome-derived inserts encoding antibiotic resistance, we found a subset of 29 that also contained genes similar (>96% nucleotide sequence identity) to previously characterized transposases (table S6). Of these, 14 transposases were identical to those previously identified on resistance and conjugative plasmids from *Bacteroides fragilis* and clinical pathogenic isolates of *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella enterica* (table S6). Interestingly, the proportion of identified transposases derived from the culturable aerobic isolates (90%) was significantly higher than those derived from metagenomic sampling (Pearson's chi-square test, $P < 0.0005$).

Nearly half of the resistance genes identified in the cultured human gut isolates were identical at the nucleotide level to resistance genes from human pathogenic isolates. Although this identity provides no information regarding the direction or mechanism of transfer, we can offer some speculation regarding the implications of our findings. First, the human microbiome may constitute a mobilizable reservoir of antibiotic resistance genes that are accessed by a pathogenic bacterium to acquire antibiotic resistance, although direct experimental proof of in vivo transfer of antibiotic resistance genes within the human microbiome remains to be shown. Second, despite selecting samples from untreated healthy humans, the aerobic cultured isolates may be dormant pathogens inhabiting the human micro-

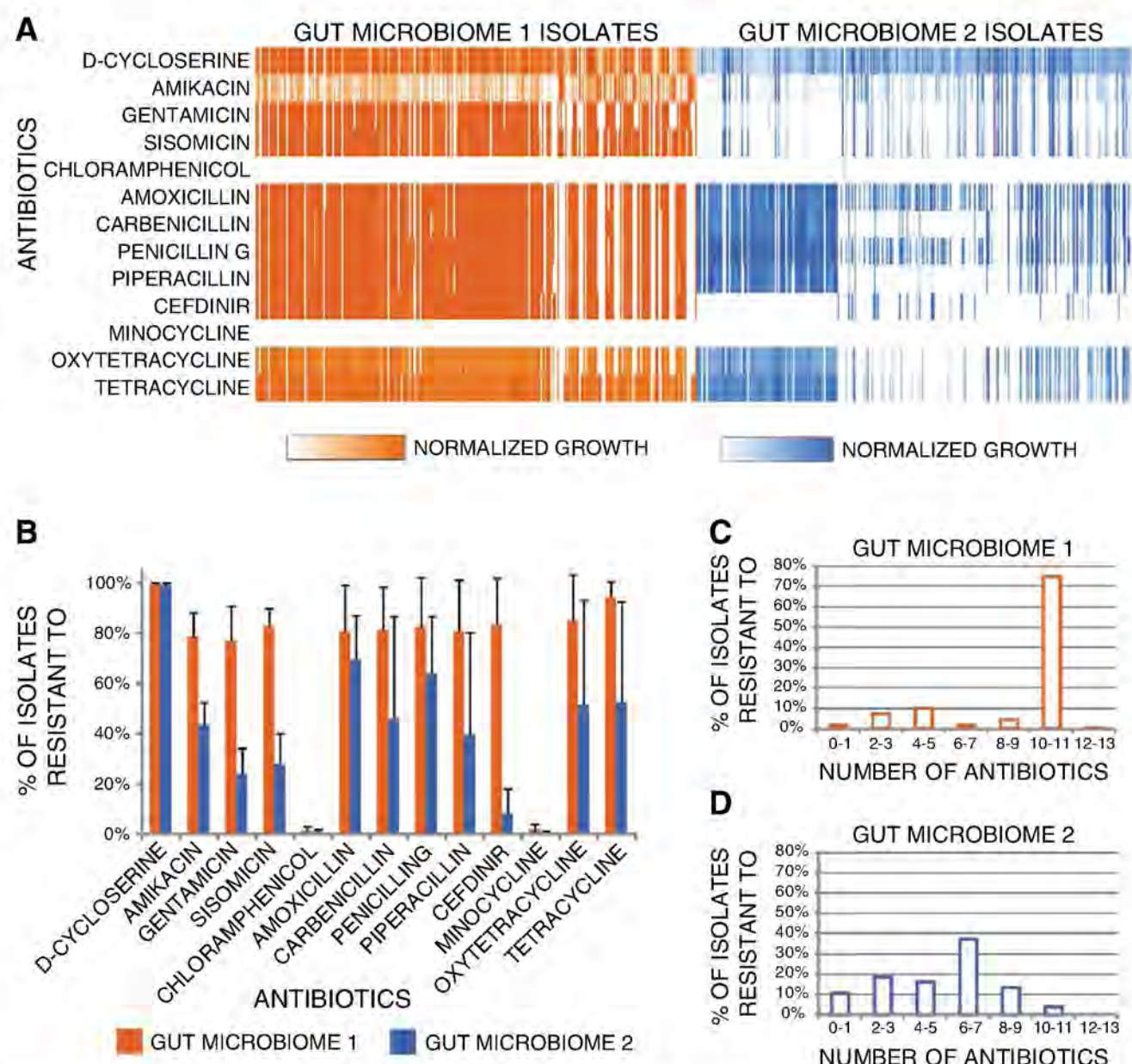


Fig. 2. Antibiotic resistance profiles of cultured aerobic gut microbiome isolates. (A) Heat map displaying resistance profiles of 572 aerobic bacterial isolates obtained across three different sampling times from two human gut microbiomes. Linear color-scaled bars display 7436 growth measurements of aerobic cultured microbiome isolates after 24 hours at 37°C in Luria broth containing one of 13 antibiotics at concentrations between 20 and 100 µg/mL that prevent the growth of wild-type *E. coli* (table S1). White denotes no growth, and color intensity is proportional to growth in the presence of antibiotic, scaled to growth in the absence of antibiotic per individual isolate. (B) Percentage of aerobic gut isolates resistant to each of 13 antibiotics. Each data point represents the mean number of isolates resistant to each antibiotic, and error bars represent the standard deviation of this mean value from each of the three sampling times. Histograms depict the distribution of the number of different antibiotics that aerobic (C) gut microbiome 1 and (D) gut microbiome 2 isolates are resistant to.

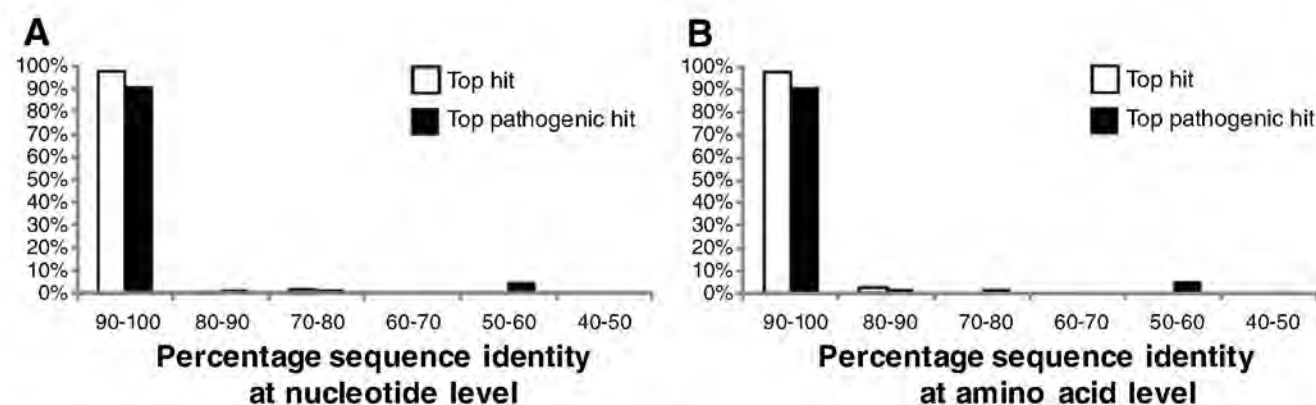


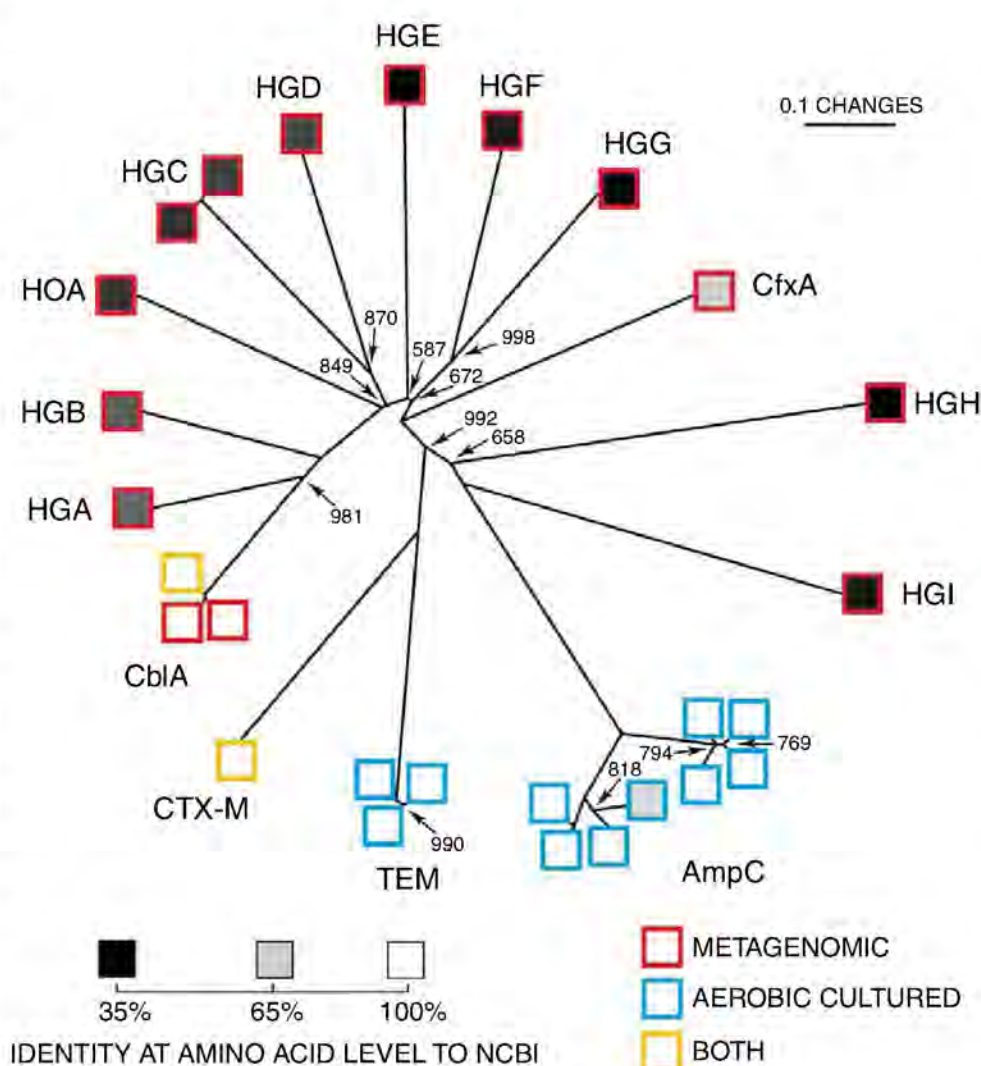
Fig. 3. Distributions of (A) nucleotide identities and (B) amino acid identities for 114 resistance genes identified from cultured aerobic gut isolates to the most similar resistance gene from any organism (white bars) as well as the most similar resistance gene harbored by a pathogenic isolate (black bars) in GenBank (table S4) (24). One resistance gene with no significant similarity to sequences in GenBank is not shown.

biome. Third, by contrast with the cultured isolates, the resistance genes discovered by the culture-independent approach were distantly related to resistance genes from even closely related pathogenic isolates, which may reflect an unappreciated barrier to lateral gene transfer in vivo between the dominant commensals in healthy humans and

disease-causing isolates. Fourth, this work exposes previous substantial undersampling of antibiotic resistance genes in the human microbiome.

We found many microbial DNA fragments encoding resistance genes that have never before been described, and our analysis suggests that we have just begun to scratch the surface of the im-

Fig. 4. The phylogenetic relationship of unique beta-lactamases derived from gut and oral microbiomes from healthy humans is displayed as an unrooted neighbor-joining tree (24). Except for nodes indicated, bootstrap values = 1000. Scale bar is in fixed amino acid substitutions per sequence position. Border colors of squares denote sequences derived from cultured aerobic gut isolates (blue), metagenomic DNA (red), or both (yellow). Internal shading of each square represents percentage amino acid identity to the most similar sequence in GenBank, with a linear gradient between 100% identity (white) and 35% identity (black). Sequence groups are labeled according to standard nomenclature (Table 1) (24, 32).



mense diversity of antibiotic resistance machinery in the human microbiome. More than half of the inserts that were derived from metagenomic libraries and libraries from cultured gut aerobes were sequenced only once in our experiment (fig. S4), and we estimate that complete sequencing of these libraries would yield hundreds more resistance genes (24). Interestingly, when we compared the resistance genes derived from the microbiomes of the two different individuals, we found that over 65% of the resistance genes derived from cultured aerobes were highly similar (>90% nucleotide sequence identity) between the two individuals, whereas less than 10% of the metagenomically derived resistance genes were highly similar between the individuals (table S7) (24).

Many commensal bacterial species, which were once considered relatively harmless residents of the human microbiome, have recently emerged as multidrug-resistant disease-causing organisms (7). In the absence of in-depth characterization of the resistance reservoir of the human microbiome, the process by which antibiotic resistance emerges in human pathogens will remain unclear.

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34. We acknowledge J. Davies, J. Aach, and M. Strong for helpful discussions regarding this manuscript; and the expert assistance of G. Jacoby and K. Bush for beta-lactamase enzyme family nomenclature, L. Kraal and G. Rockwell for sequence manipulations and similarity computations, and S. Caliri, A. Ellison, and T. Ellison for microbial culturing and DNA processing. We acknowledge National Human Genome Research Institute Centers of Excellence in Genomic Science, Personal Genome Project, Bill and Melinda Gates Foundation, Harvard Biophysics Program, Hartmann Foundation, and Det Kongelige Danske Videnskabsnernes Selskab for funding. GenBank accession numbers GQ342978 to GQ343187 are listed in Table 1 and tables S3, S4, and S7. M.O.A.S. and G.M.C. advise many companies, listed in (24); none is working within fields related to the subject of the manuscript.

Supporting Online Material

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Materials and Methods

Figs. S1 to S7

Tables S1 to S7

References

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Motile Cilia of Human Airway Epithelia Are Chemosensory

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Cilia are microscopic projections that extend from eukaryotic cells. There are two general types of cilia; primary cilia serve as sensory organelles, whereas motile cilia exert mechanical force. The motile cilia emerging from human airway epithelial cells propel harmful inhaled material out of the lung. We found that these cells express sensory bitter taste receptors, which localized on motile cilia. Bitter compounds increased the intracellular calcium ion concentration and stimulated ciliary beat frequency. Thus, airway epithelia contain a cell-autonomous system in which motile cilia both sense noxious substances entering airways and initiate a defensive mechanical mechanism to eliminate the offending compound. Hence, like primary cilia, classical motile cilia also contain sensors to detect the external environment.

Cilia can be divided into two general types, primary and motile (1–3). Primary cilia are sensory organelles that contain a variety of receptors, including G protein-coupled receptors (1). Cells typically sprout a single primary cilium with a characteristic 9+0 cytoskeletal

variety of receptors, including G protein-coupled receptors (1). Cells typically sprout a single primary cilium with a characteristic 9+0 cytoskeletal

structure (axoneme). In contrast, motile cilia can occur in the tens and hundreds on epithelial cells, have a typical 9+2 axoneme, and serve a mechanical role (1–3). Although their functions differ, there are exceptions to the primary cilia–motile cilia distinction (3); for example, primary nodal cilia exhibit rotational movement (4, 5). In addition, the two types of cilia share many structural and molecular features (2, 6); they are evolutionarily related (7); and genetic diseases such as Bardet-Biedl Syndrome can affect both (8–10). However, whether classic motile cilia can also function as sensory organelles is not clear.

On airway epithelia, motile cilia move mucus out of the lung, and their disruption causes airway disease (11, 12). Because defense of the airway involves detection of danger signals, we hypothesized that motile cilia might sense noxious stimuli. To test this hypothesis, we searched for sensory-related genes in microarray expression data from primary cultures of differentiated human airway epithelia (13). Microarray analysis (14) and reverse transcription–polymerase chain reaction (RT-PCR) revealed that the epithelia expressed several members of the bitter taste receptor (T2R) family (Fig. 1, A and B). The human genome contains ~25 T2R genes. Several T2Rs may detect more than one different bitter compound, and individual taste receptor cells express multiple T2Rs (15, 16). Thus, in the tongue, the system is broadly tuned to detect multiple bitter compounds and warn against ingestion of toxic substances.

We studied several T2Rs for which antibodies are available and/or for which ligands are known, and we identified T2R4, T2R43, T2R38, and T2R46 in human airway epithelia (Fig. 2, A to C, and figs. S1 to S6). Only ciliated epithelial cells expressed these receptors, and they specifically localized in cilia, which were identified by acetylated α -tubulin immunostaining. Almost every ciliated cell expressed each of the four T2Rs, although the intensity varied from cell to cell and in some ciliated cells T2R43 was not detected (Fig. 2B). Thus, single ciliated epithelial cells express multiple T2Rs, similar to individual taste receptor cells that express multiple T2Rs (17). The receptors appeared to localize preferentially at different places along the cilium. For example, T2R4 seemed to sit nearer the tips of cilia, whereas T2R43 appeared to reside more proximally in cilia (Fig. 2, A and B, and figs. S1, S3, and S7), which was more apparent

Fig. 1. Bitter taste receptors are expressed in human airway epithelia. (A) Microarray analysis of mRNA isolated from airway epithelial samples isolated from eight human donors (14, 31). Data are the mean $\pm$ SEM. (B) RT-PCR analysis of mRNA isolated from differentiated cultures of human airway epithelia.

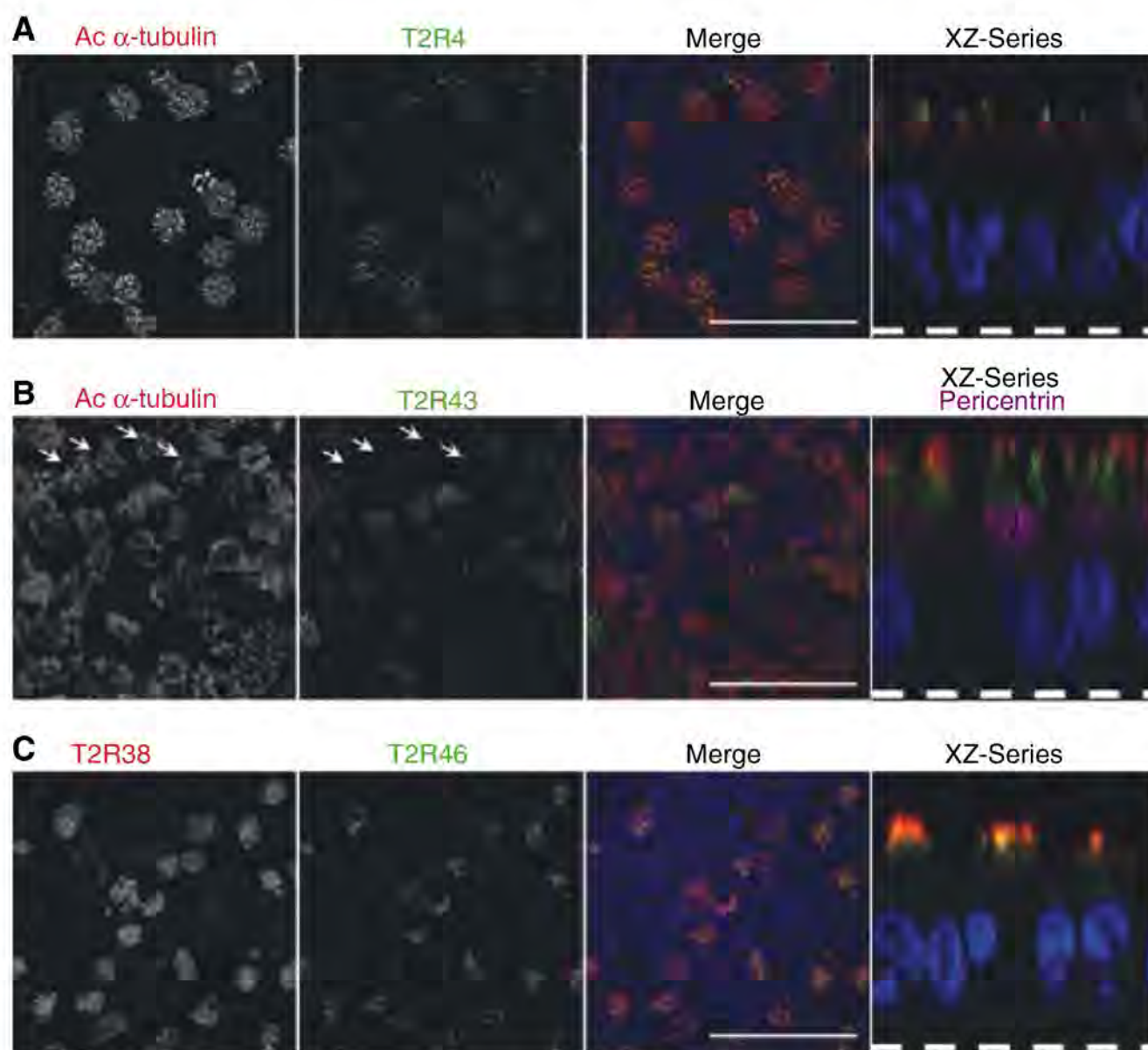
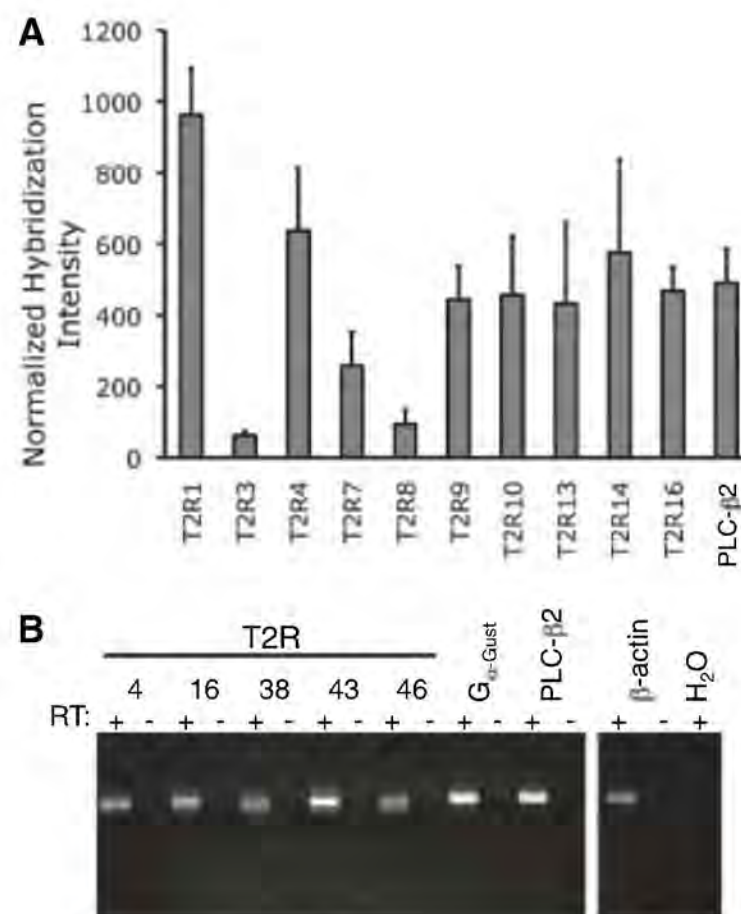


Fig. 2. T2Rs localize along the motile cilia of airway epithelia. Confocal immunofluorescence microscopy of cultured human airway epithelia with antibodies to T2R (anti-T2R) showed that (A) T2R4 and (B) T2R43 (both green) localize to motile cilia, which were identified by antibodies to acetylated α -tubulin (red). Nuclei stained with DAPI (4',6-diamidino-2-phenylindole) are in blue. Arrows in (B) indicate the location of ciliated cells that were not labeled by anti-T2R43. (C) T2R38 (red) and T2R46 (green) localize to motile cilia. Data are stacks of confocal z-series images in the X-Y plane and a single X-Z plane image (right; the dashed line indicates the filter). (B) (right) also shows antibodies to pericentrin (purple), which label the basal body below the cilia. Scale bars, 20 μ m. See also figs. S1 to S7.

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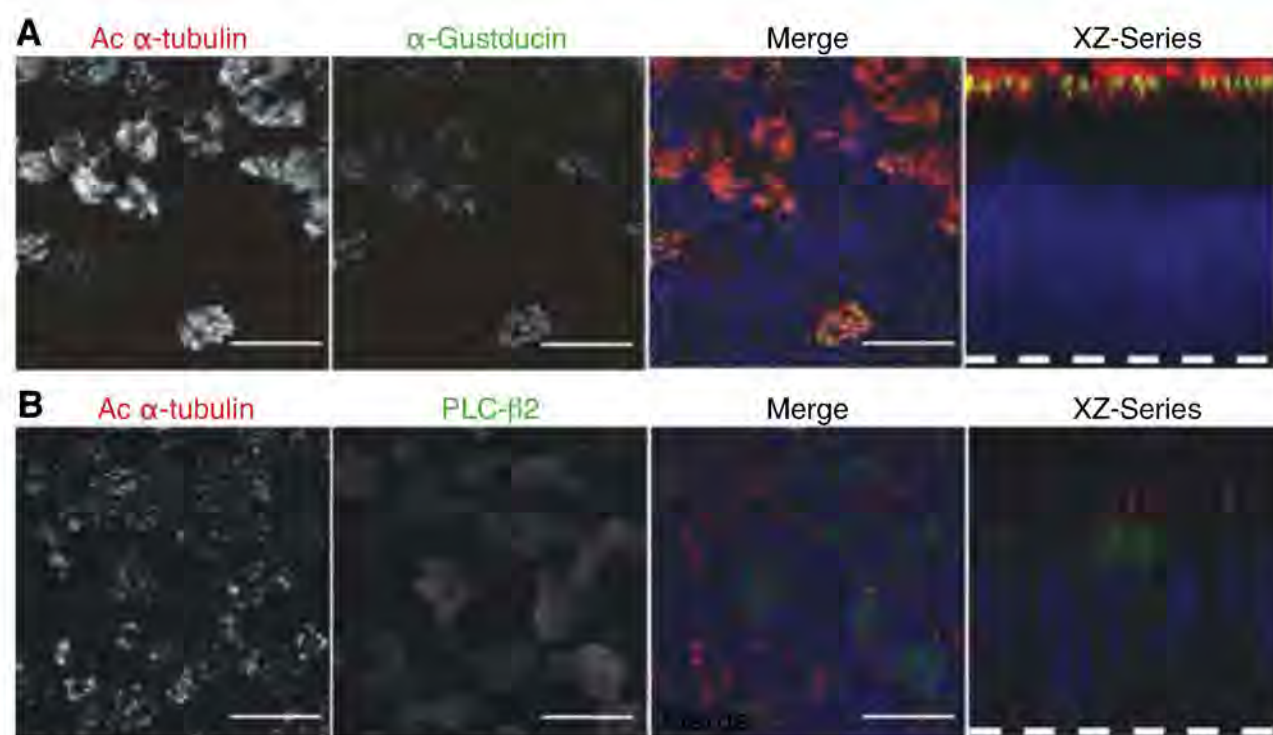


Fig. 3. Components of the bitter taste transduction pathway localize to ciliated cells and to the cilia. Immunolocalization of (A) α -gustducin and (B) PLC- β 2 are in green and acetylated α -tubulin is in red. Other aspects of the figure are as described in the legend to Fig. 2. Scale bars, 20 μ m. See also figs. S1 to S3.

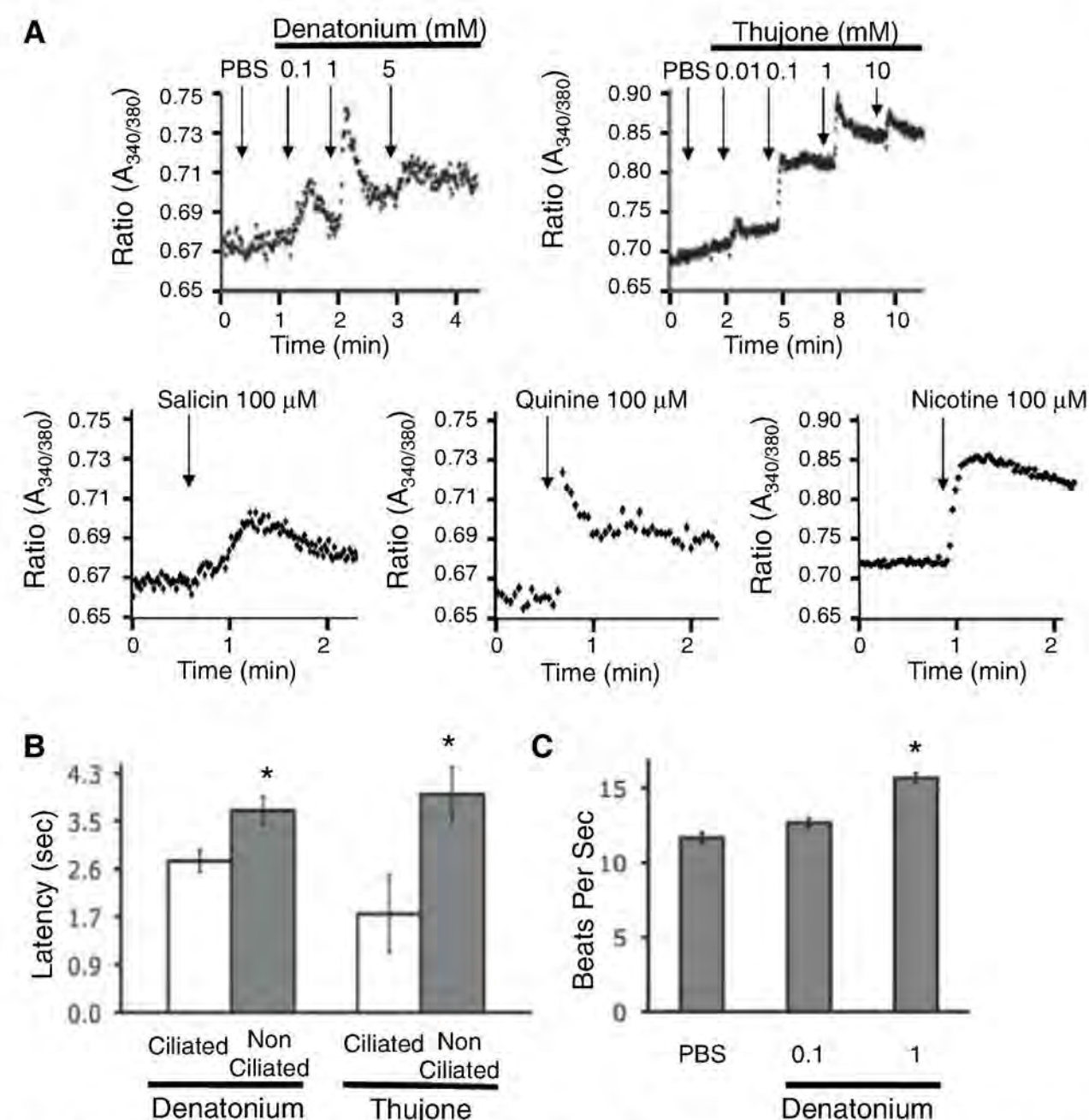


Fig. 4. Bitter compounds increase $[Ca^{2+}]_i$ in ciliated cells. (A) Differentiated airway epithelia were loaded with Fura2-AM and fluorescence imaging was used to assess changes in $[Ca^{2+}]_i$, indicated as the ratio of emission when excited at 340 nm versus 380 nm ($A_{340/380}$). Denatonium, thujone, salicin, quinine, or nicotine was added as indicated. "PBS" indicates addition of phosphate-buffered saline and arrows indicate the time of addition. Data points are the average of 20 cells. (B) Latency to increase in $[Ca^{2+}]_i$ after addition of 1 mM denatonium or 1 mM thujone; latency includes the time for the compound to diffuse to the cell after its addition. $*P < 0.001$, $N = 20$. See also fig. S8. (C) Denatonium increases ciliary beat frequency. Data are the ciliary beat frequency in differentiated human airway epithelia treated with PBS or denatonium. $N = 4$, $*P < 0.01$. See also figs. S8 and S9. The error bars in (B) and (C) denote SEM.

when we immunostained both T2R4 and T2R43 in the same epithelia (fig. S7). Co-labeling T2R38 and T2R46 revealed a similar relation, with T2R38 appearing to sit more distally than T2R46 (Fig. 2C and fig. S7).

The T2R signal transduction pathway comprises several proteins, including the G protein α -gustducin and the enzyme phospholipase C- β 2 (PLC- β 2) (15, 16). We detected their transcripts in airway epithelia (Fig. 1B). Immunostaining revealed these signaling molecules only in ciliated epithelial cells (Fig. 3, A and B, and fig. S3). Like the T2Rs, α -gustducin resided in cilia. In contrast, PLC- β 2 appeared to sit below the cilia in the apical portion of the cell. We speculate that the seeming complexity of T2R and signaling protein localization along the ciliary shaft and in the cell might provide a mechanism for coupling to other signaling pathways or influencing cilia function. Distinct localization may also occur with other proteins that are localized proximally in cilia and flagella (18, 19).

To determine whether the T2R signaling pathway was functional, we applied bitter compounds to differentiated human airway epithelia and measured changes in the T2R-associated second messenger, intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. Denatonium is a very bitter compound and a ligand for T2R4 (15). Its application induced transient, dose-dependent increases in $[Ca^{2+}]_i$ (Fig. 4A). Several other bitter compounds, including thujone (activates T2R14), salicin (activates T2R16), quinine, and nicotine, also elicited increases in $[Ca^{2+}]_i$. The denatonium and thujone concentrations inducing increases in $[Ca^{2+}]_i$ were in the same range as those that elicit responses in heterologous cells expressing T2Rs (20).

Airway epithelia contain both ciliated and nonciliated cells, and when $[Ca^{2+}]_i$ increases in an individual airway epithelial cell, it can rapidly spread through gap junctions to adjacent cells (21). Thus, the $[Ca^{2+}]_i$ increase shown in Fig. 4A could have begun from either cell type. Therefore, we tested if $[Ca^{2+}]_i$ first increased in ciliated or nonciliated cells by applying the bitter compound apically and comparing the latency to an increase in $[Ca^{2+}]_i$ in the different cells. After addition of denatonium or thujone, $[Ca^{2+}]_i$ rose in ciliated cells before increasing in other cells (Fig. 4B), consistent with our finding that only ciliated cells bear receptors for bitter compounds. We also studied cells growing on glass in short-term culture because the $[Ca^{2+}]_i$ increase does not spread; in those cells, denatonium increased $[Ca^{2+}]_i$ only in ciliated cells (fig. S8).

We assessed the functional consequences of activating T2Rs in airway epithelia by measuring the frequency of cilia beating. Because an elevated $[Ca^{2+}]_i$ can stimulate ciliary motility (22), we predicted that a bitter compound would increase frequency. Indeed, applying denatonium increased ciliary beat frequency $\sim 25\%$ (Fig. 4C and fig. S9).

Thus, some of the same receptors that detect noxious compounds on the tongue to protect animals from ingesting harmful material may also defend human airways. The receptors are positioned on cilia, a location optimal for sampling air and material entering the lung. Moreover, the bitter compound denatonium stimulated ciliary activity, which should hasten elimination of noxious and harmful substances. Substances that activate T2Rs might enter airways through inhalation or aspiration or be generated within the lung by infection. We speculate that this signaling system might also play a role in airway disease. For example, in cystic fibrosis, lungs are commonly infected with *Pseudomonas aeruginosa*. This bacterium produces quorum-sensing molecules that are lactones, which activate some bitter taste receptors (23, 24). Airway T2Rs might also be activated by cigarette smoke, which contains the bitter-tasting compound nicotine (25). In addition, airway cilia are lost in some viral infections and with cigarette smoking (26), which would disrupt this defensive system.

Previous work suggested general roles for the two types of vertebrate cilia—primary cilia are sensory and motile cilia are mechanical. Studies of primary nodal cilia in mouse embryos indicated that the distinction is not absolute; those cilia can exhibit a rotational movement different from the planar beating typical of motile cilia (4, 5). In addition, proteomic studies of *Chlamydomonas* flagella identified numerous proteins involved in signal transduction (27). Our present data indicate that classical motile cilia also have a sensory function.

T2Rs on taste receptor cells, which are not known to have primary cilia, detect bitter ligands and transmit that information to nerves to

elicit a response (15, 20). In cells outside the tongue where α -gustducin and T2Rs have been identified—nasal and laryngeal solitary chemosensory cells and intestinal tract enteroendocrine cells—signals are transmitted to the associated nerve networks (28–30). In contrast, in airway epithelia, T2Rs enable cell-autonomous detection of a signal followed by a response in the same cell to eliminate harmful substances.

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Supporting Online Material

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Figs. S1 to S9

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The E3 Ligase TRAF6 Regulates Akt Ubiquitination and Activation

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Akt signaling plays a central role in many biological functions, such as cell proliferation and apoptosis. Because Akt (also known as protein kinase B) resides primarily in the cytosol, it is not known how these signaling molecules are recruited to the plasma membrane and subsequently activated by growth factor stimuli. We found that the protein kinase Akt undergoes lysine-63 chain ubiquitination, which is important for Akt membrane localization and phosphorylation. TRAF6 was found to be a direct E3 ligase for Akt and was essential for Akt ubiquitination, membrane recruitment, and phosphorylation upon growth-factor stimulation. The human cancer-associated Akt mutant displayed an increase in Akt ubiquitination, in turn contributing to the enhancement of Akt membrane localization and phosphorylation. Thus, Akt ubiquitination is an important step for oncogenic Akt activation.

Protein ubiquitination is an important post-translational modification that regulates various biological functions (1, 2). Al-

though ubiquitination often results in protein degradation, a certain type of ubiquitination is important for signaling activation and protein

trafficking (1, 2). Ubiquitination through Lys⁴⁸ (K48) (3) of the ubiquitin chain generally targets proteins for degradation, whereas ubiquitination through K63 plays a critical role in signaling activation and protein trafficking (1, 2). Akt (also known as protein kinase B) is an important component of cell signaling pathways that regulate cell survival and metabolism. Although membrane recruitment of Akt by growth-factor stimuli is a critical step for Akt phosphorylation, it is not clear how Akt is recruited to the plasma membrane. Because ubiquitination can regulate protein trafficking (1), we tested whether Akt is ubiquitinated in cells. Akt was ubiquitinated in the absence of

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proteasome inhibitor MG132 (fig. S1A). Ubiquitination occurred through K63 but not through K48 (Fig. 1A). Akt ubiquitination could be modified by K11-linked ubiquitin but not through K6 and K48 linkages (fig. S1B).

We screened a panel of E3 ubiquitin ligases for Akt ubiquitination. Although Mdm2 E3 ligase interacts with Akt (4–6), it failed to promote Akt ubiquitination (fig. S2A), as did c-IAP1, c-IAP2, Cbl-b, Itch, Smurf2, and Fbw7 (fig. S2A). Overexpression of c-IAP1 and c-IAP2 reduced Akt ubiquitination (fig. S2, A and B). Because Akt underwent K63 ubiquitination, we focused on TRAF6 and HectH9, two E3 ubiquitin ligases that catalyze K63 ubiquitination and function in Toll-like receptor (TLR) or interleukin-1 (IL-1) signaling and oncogenic activation of Myc, respectively (7–9). TRAF6 promoted Akt ubiquitination, whereas HectH9 did not (Fig. 1B).

Activity of Akt was not required for TRAF6-mediated ubiquitination (fig. S2C). However, TRAF6 E3 ligase activity was required. The

TRAF6 C⁷⁰→A⁷⁰ [C70A (3)] mutant, which loses E3 ligase activity (9), had compromised activity (Fig. 1C), which was not due to a defect in Akt interaction (fig. S3A). Although TRAF6 induced Akt ubiquitination, it did not decrease the abundance of Akt (Fig. 1, B and C), suggesting it does not mediate K48 ubiquitination. Indeed, TRAF6 promoted K63 ubiquitination of Akt but not K48 ubiquitination (Fig. 1D). TRAF6, but not TRAF6 C70A, induced Akt ubiquitination in vitro (Fig. 1E and fig. S2D). These results suggest that TRAF6 is an E3 ubiquitin ligase for Akt.

Coimmunoprecipitation experiments revealed that Akt interacted with overexpressed TRAF6 and with TRAF6 C70A (fig. S3A). We detected interaction between endogenous Akt and TRAF6 in cells stimulated with insulin-like growth factor-1 (IGF-1) or IL-1 β (fig. S3B), both of which activate Akt signaling. Glutathione-S-transferase (GST)-tagged TRAF6 interacted with Akt directly in vitro (fig. S3C). Ubiquitination of the Akt1 and Akt2 isoforms, but

not that of Akt3, was induced by TRAF6 (fig. S4A). Coimmunoprecipitation assay revealed that TRAF6 interacted with all Akt isoforms (fig. S4B).

Overexpression of TRAF6, but not that of TRAF6 C70A, enhanced phosphorylation of Akt at T308 (3) but not at S473, and this enhancement was accompanied by increased Akt activity toward glycogen synthase kinase 3 β (GSK3 β) (Fig. 1F) (10, 11). A control E3 ligase, HectH9, failed to regulate phosphorylation of Akt (fig. S5). Overexpression of c-IAP1 and c-IAP2, which slightly inhibited ubiquitination of Akt, reduced phosphorylation of Akt on T308 (fig. S2B). Thus, TRAF6 may enhance Akt phosphorylation by promoting Akt ubiquitination.

We compared Akt ubiquitination and phosphorylation in *Traf6*^{+/+} and *Traf6*^{-/-} primary mouse embryonic fibroblasts (MEFs) treated with activators of Akt. Upon IGF-1 treatment, endogenous Akt ubiquitination was reduced in *Traf6*^{-/-} MEFs compared with that in *Traf6*^{+/+}

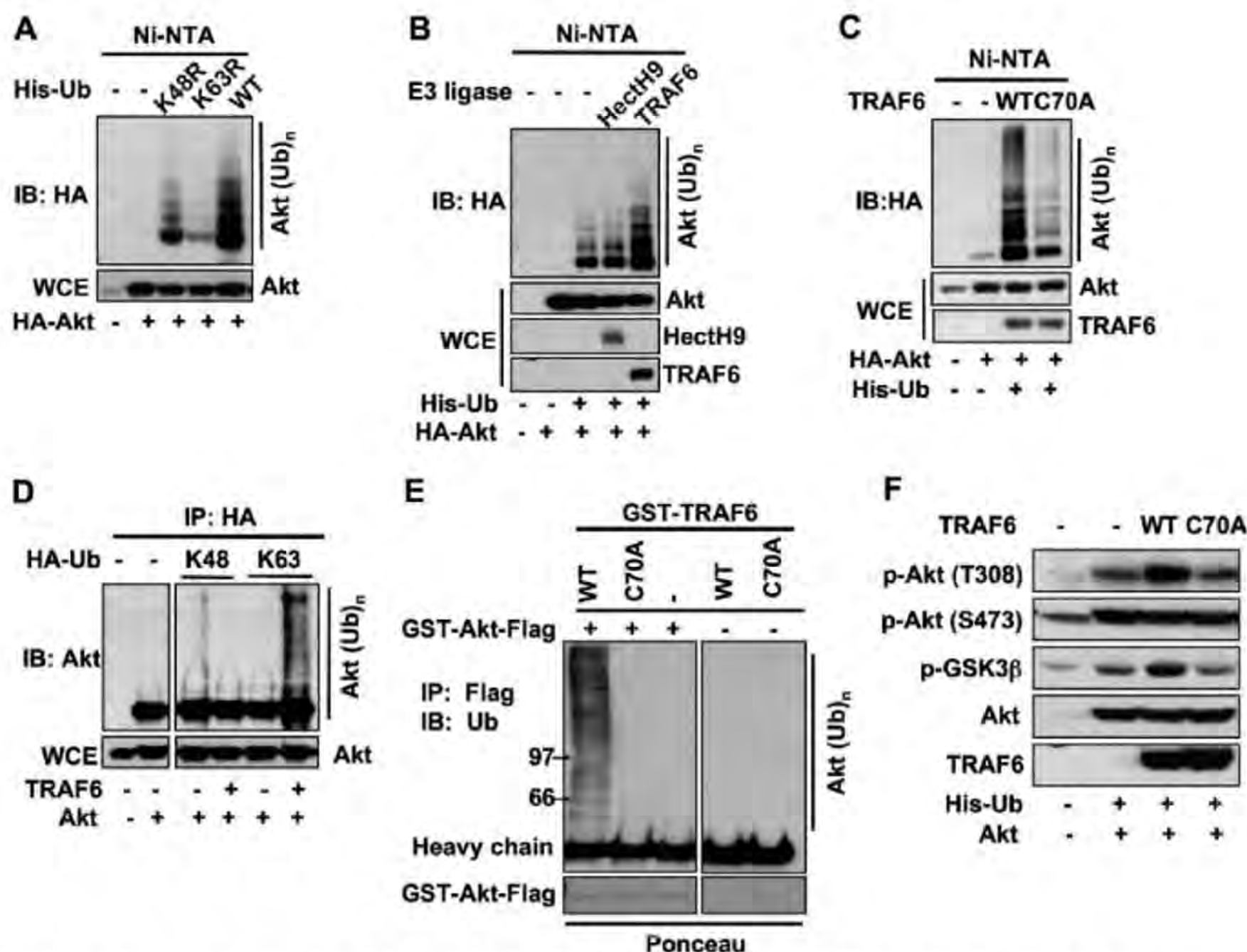


Fig. 1. TRAF6 is an E3 ubiquitin ligase for Akt. (A) Immunoblot (IB) of lysed 293T cells transfected with Akt along with His-ubiquitin (His-Ub), His-Ub K48R, or His-Ub K63R constructs. WT indicates wild type; Ni—nitrilotriacetic acid (NTA), nickel bead precipitate; and WCE, whole-cell extracts. (B) IB of lysed 293T cells transfected with hemagglutinin (HA)-Akt and His-Ub, along with various E3 ligases. (C) IB of lysed 293T cells transfected with HA-Akt and His-Ub, along

with TRAF6 or TRAF6 C70A. (D) IB of lysed 293T cells transfected with Akt and TRAF6, along with HA-Ub K48 (K48-only ubiquitin) or HA-Ub K63 (K63-only ubiquitin). IP, immunoprecipitation. (E) GST-Akt-Flag proteins were incubated with adenosine triphosphate, E1, and E2 along with GST, GST-TRAF6, or GST-TRAF6 C70A proteins for in vitro ubiquitination of Akt. (F) WCE from 293T cells transfected with indicated plasmids was collected for IB analysis.

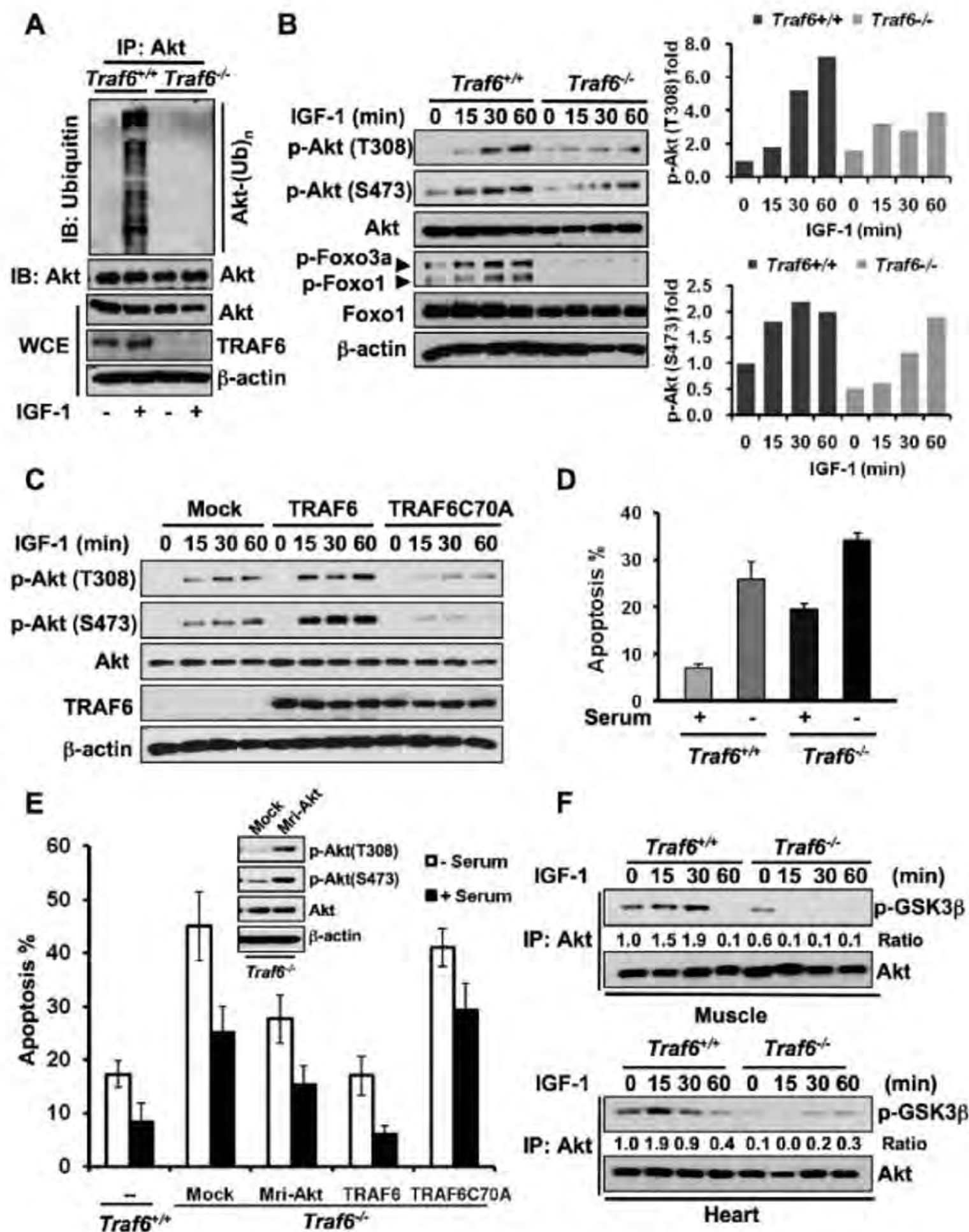
MEFs (Fig. 2A). Similarly, Akt ubiquitination was induced by 10% fetal bovine serum (FBS) or IL-1 β in *Traf6*^{+/+} MEFs but not in *Traf6*^{-/-} MEFs (fig. S6A). Consequently, Akt phosphorylation at T308 and S473 after IGF-1 treatment was reduced in *Traf6*^{-/-} MEFs compared with that in *Traf6*^{+/+} MEFs (Fig. 2B). This defect in Akt phosphorylation in *Traf6*^{-/-} MEFs was correlated with an impairment in phosphorylation of Foxo1 and Foxo3a, two Akt substrates (10, 11). Ubiquitination of TRAF6 was also induced by IGF-1 stimulation (fig. S7A). TRAF6 interacted with IGF-1 receptor (IGF-1R) in serum-free conditions but not after

IGF-1 treatment (fig. S7B). Thus, activated IGF-1 receptor may directly engage TRAF6 activation.

Phosphorylation of Akt T308 and S473 was also inhibited in *Traf6*^{-/-} MEFs treated with 10% FBS (fig. S6B). In contrast, phosphorylation of ERK1 and ERK2 was comparable in wild-type and *Traf6*^{-/-} MEFs (fig. S6B), suggesting that TRAF6 selectively affects Akt activation. IL-1 and lipopolysaccharide (LPS) activate the IL-1 receptor (IL-1R) and Toll-like receptor 4 (TLR4), respectively. Because TRAF6 is a critical regulator for both IL-1R and TLR4 signaling (8, 9), we tested whether

IL-1 β -induced and LPS-induced phosphorylation of Akt acts through TRAF6. LPS and IL-1 β induced phosphorylation of Akt at T308 in *Traf6*^{+/+} MEFs in the presence of 10% FBS; this effect was reduced in *Traf6*^{-/-} MEFs (fig. S6, C and D). Neither LPS nor IL-1 β was sufficient to trigger phosphorylation of Akt at either T308 or S473 in serum-starved conditions (0.1% FBS) in multiple primary cell lines (fig. S8), suggesting that IL-1 β and LPS may require cooperation with growth-factor receptor signaling to induce Akt activation. Restoration of TRAF6 expression in *Traf6*^{-/-} MEFs rescued the defect of Akt phosphorylation in

Fig. 2. TRAF6 is required for ubiquitination and phosphorylation of Akt. **(A)** *Traf6*^{+/+} and *Traf6*^{-/-} MEFs were serum-starved and treated with or without IGF-1 for 30 min; WCE were collected for immunoprecipitation with Akt, followed by IB analysis. **(B)** MEFs were serum-starved, treated with IGF-1 for various time points, and harvested for IB analysis. The quantification results are shown on the graphs to the right. **(C)** Primary *Traf6*^{-/-} MEFs infected with mock, TRAF6, or TRAF6 C70A mutant were treated with IGF-1 at various time points and harvested for IB analysis. **(D)** MEFs were cultured in 10% FBS or serum-starved for 2 days, and apoptosis was determined by annexin V staining, followed by flow cytometry analysis. Results are presented as mean values $\pm$ SD. **(E)** Primary MEFs were infected with mock, constitutively active Akt (Mri-Akt), TRAF6, or TRAF6 C70A; and apoptosis and IB analysis were determined. Results are presented as mean values $\pm$ SD. **(F)** Heart and skeletal muscles isolated from WT and *Traf6*^{-/-} mice ($n = 4$) injected with IGF-1 at various time points were subjected to an in vitro Akt kinase assay and IB analysis.



cells stimulated with IGF-1 or IL-1 β , whereas restoration of TRAF6 C70A did not (Fig. 2C and fig. S6E). Thus, TRAF6 is critical for Akt phosphorylation and activation through induction of Akt ubiquitination.

Because Akt influences cell survival and apoptosis (10, 11), we tested whether *Traf6* deficiency sensitized cells to apoptosis after serum withdrawal. Apoptosis in *Traf6*^{-/-} MEFs was higher than in *Traf6*^{+/+} MEFs in the presence and absence of serum (Fig. 2D and fig. S9). The active, cleaved form of caspase 3, a mediator of apoptosis, was more abundant in *Traf6*^{-/-} MEFs than in *Traf6*^{+/+} MEFs (fig. S10A). Res-

toration of TRAF6, but not TRAF6 C70A, rescued *Traf6*^{-/-} MEFs from apoptosis (Fig. 2E). A constitutively active form of Akt partially rescued cells from apoptosis (Fig. 2E). Thus, other signaling pathways may be also involved. Because apoptosis inducers such as DNA damage agents induce phosphorylation and activation of Akt (12, 13), we tested whether TRAF6 was also involved. Doxorubicin (Dox)- and cisplatin (Cis)-induced phosphorylation of Akt T308 in wild-type MEFs was impaired in *Traf6*^{-/-} MEFs (fig. S10B). The impairment of Akt phosphorylation was correlated with increased caspase 3 activation in *Traf6*^{-/-} MEFs (fig. S10C).

TRAF6 is expressed in most mouse tissues (14, 15). We compared Akt activity in skeletal muscle and heart tissues from wild-type and *Traf6*^{-/-} mice. Steady-state Akt activity in heart muscle, but not skeletal muscle, was lower in *Traf6*^{-/-} mice than in wild-type mice (fig. S11). Akt activation in animals injected with IGF-1 was impaired in both forms of muscles in *Traf6*^{-/-} mice (Fig. 2F). These results suggest that TRAF6 plays a critical role in Akt activation in vivo.

Because the pleckstrin homology (PH) domain of Akt is critical for phosphatidylinositol (3,4,5)-trisphosphate (PIP3) lipid binding and protein-protein interaction (16, 17), we analyzed whether the PH domain of Akt influenced TRAF6-mediated ubiquitination of Akt. TRAF6 failed to promote ubiquitination of the Akt mutant devoid of the PH domain (Fig. 3A). Of the six lysine residues within the PH domain of Akt, mutation on either K8 or K14 to arginine (R) most substantially reduced Akt ubiquitination and Akt phosphorylation at T308 and S473 (Fig. 3B). The K8 and K14 residues are well conserved from *Drosophila* to humans (fig. S12), suggesting that the ubiquitination of Akt may be evolutionarily conserved.

Akt K8R bound effectively to isolated PIP3, but Akt K14R did not (Fig. 3C). The K14 residue lies within the PIP3 lipid-binding pocket (18–20). Overexpression of TRAF6 did not enhance the binding of Akt to PIP3 (Fig. 3C). Thus, Akt ubiquitination by TRAF6 appears not to influence PIP3 lipid binding, and the defect in phosphorylation of Akt K8R is not due to its impairment in PIP3 binding.

A mutation in the PH domain [E17 $\rightarrow$ K17, E17K (3)] of Akt has been identified in human cancer patients, including those with breast cancer (19). This cancer-associated Akt mutant exhibited constitutive Akt phosphorylation at T308 but not at S473 and had greater oncogenic potential. Basal ubiquitination of the E17K mutant was much higher than that of wild-type Akt (Fig. 3D). Overexpression of TRAF6 still increased ubiquitination of this mutant but to a lesser extent than wild-type Akt (Fig. 3D). The E17K mutant displayed higher Akt phosphorylation at T308 but not at S473 (19), and this was not increased by TRAF6 overexpression (Fig. 3D). Ubiquitination of a K8R/E17K Akt mutant in vivo was reduced and correlated with a reduction in phosphorylation of Akt T308 (Fig. 3E). Thus, increased Akt ubiquitination apparently contributes to the hyperactivation of Akt observed in the Akt E17K mutant.

Because K63 ubiquitination regulates protein trafficking, we tested whether TRAF6 influenced membrane recruitment of Akt. Overexpression of TRAF6 increased Akt membrane localization, which correlated with an increase in phosphorylation and ubiquitination of Akt (Fig. 3A). IGF-1-induced Akt membrane localization and T308 phosphorylation in wild-type MEFs was abolished in *Traf6*^{-/-} MEFs (Fig. 4B).

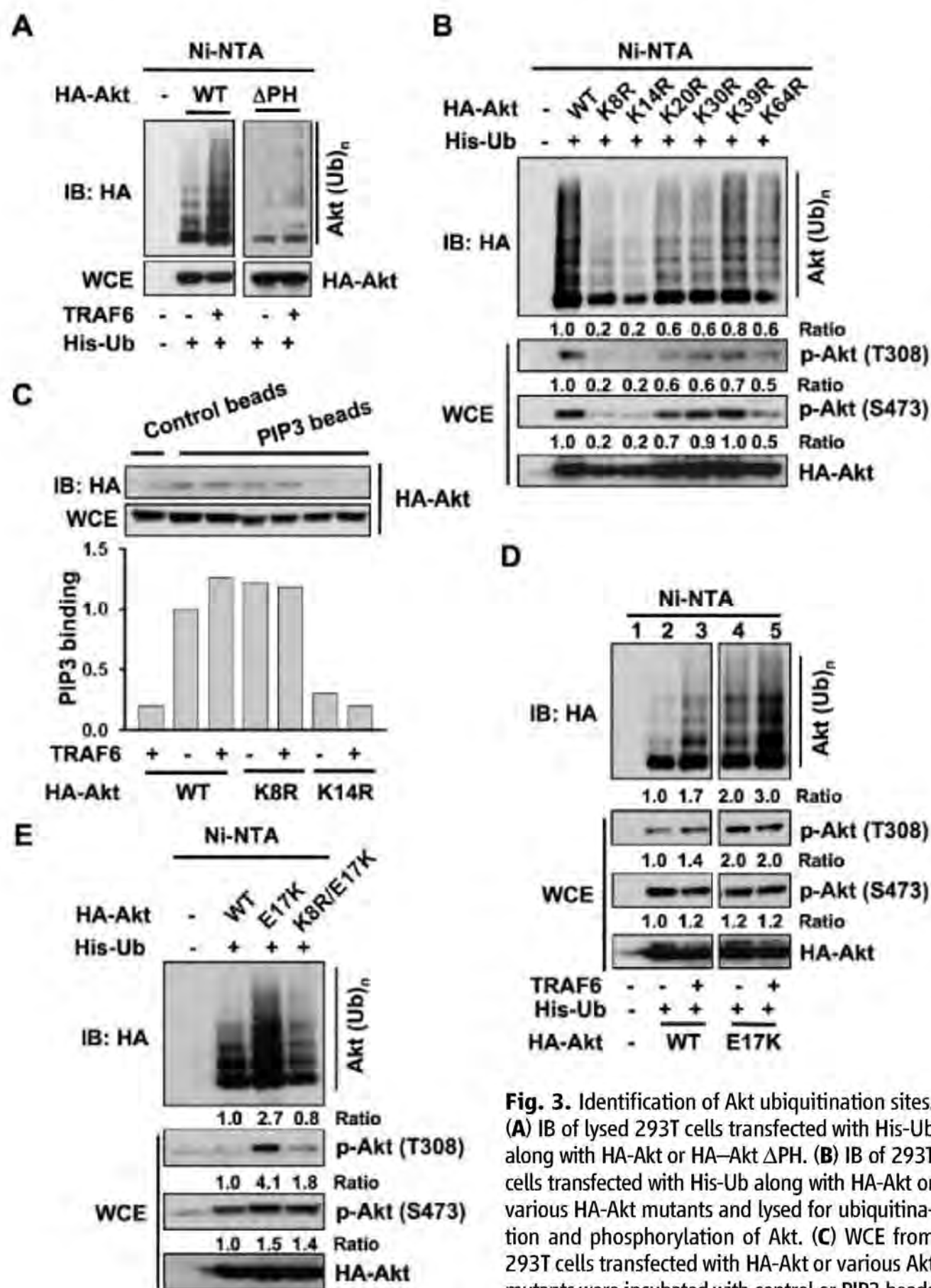


Fig. 3. Identification of Akt ubiquitination sites. (A) IB of lysed 293T cells transfected with His-Ub along with HA-Akt or HA-Akt Δ PH. (B) IB of 293T cells transfected with His-Ub along with HA-Akt or various HA-Akt mutants and lysed for ubiquitination and phosphorylation of Akt. (C) WCE from 293T cells transfected with HA-Akt or various Akt mutants were incubated with control or PIP3 beads for overnight, washed, and subjected to IB analysis. (D and E) IBs of lysed 293T cells transfected with indicated plasmids.

The PIP3 binding was calculated as the ratio between amounts of Akt bound with PIP3 beads and total amounts of Akt. (D and E) IBs of lysed 293T cells transfected with indicated plasmids.

Thus, TRAF6 is required for Akt membrane recruitment and phosphorylation upon IGF-1 stimulation.

We also compared the membrane recruitment of wild-type Akt and Akt mutants (K8R and K14R), which are defective in Akt ubiquitination. Membrane recruitment of Akt K8R and K14R upon IGF-1 treatment was reduced (Fig. 4C and fig. S13). The Akt E17K mutant localized to the membrane, even without IGF-1 stimulation, although IGF-1 stimulation further increased membrane recruitment (Fig. 4C and fig. S13).

The Akt K8R/E17K mutant showed impaired association with the membrane (Fig. 4C and fig. S13). This suggests that Akt ubiquitination contributes to membrane recruitment and phosphorylation of Akt.

Because deregulated Akt can contribute to cancer development (21, 22), we depleted TRAF6 in PC-3 prostate cancer cells by using short hairpin RNAs (shRNAs). Depletion of TRAF6 reduced Akt phosphorylation at T308 and S473 (Fig. 4D). In cells treated with IGF-1, Akt phosphorylation in TRAF6 knockdown

cells was impaired (Fig. 4E). In xenograft tumor models, the two stable TRAF6 knockdown cells had lower tumorigenic potential than control cells (Fig. 4F and fig. S14). Thus, TRAF6 appears to influence tumorigenesis in this model.

TRAF6 has an important role in TLR signaling and the innate immune response. Our results expand its known function to include the survival and oncogenic signaling pathways (fig. S15). We suggest that TRAF6 may be a previously uncharacterized oncogene that may serve as an important therapeutic target for human cancers.

References and Notes

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Figs. S1 to S15

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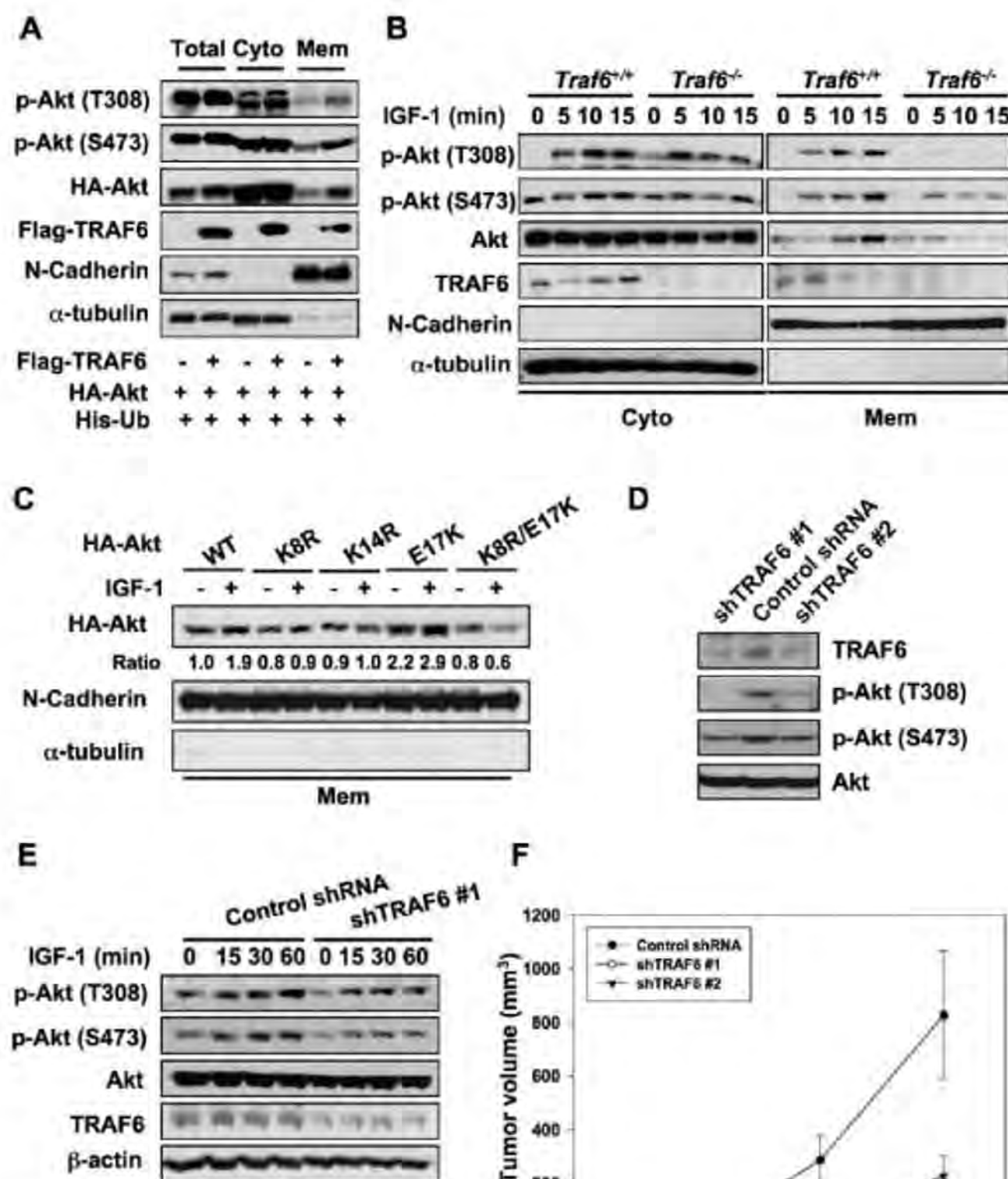


Fig. 4. Ubiquitination of Akt is required for membrane recruitment of Akt. (A) The membrane (Mem) and cytosolic (Cyto) fractions from 293T cells transfected with mock or TRAF6 were subjected to IB analysis. (B) MEFs were serum-starved and treated with IGF-1, and the membrane and cytosolic fractions were isolated for IB analysis. (C) COS-1 cells were transfected with indicated plasmids, serum-starved, and treated with IGF-1 for 15 min; and the membrane fractions were isolated for IB analysis. (D) PC-3 cells silenced with control or TRAF6 shRNAs were harvested for IB analysis. (E) PC-3 cells silenced with control or TRAF6 shRNA were serum-starved, treated with IGF-1 for various times, and harvested for IB analysis. (F) PC-3 cells silenced with control or TRAF6 shRNAs were injected into nude mice ($n = 6$ for each group) and monitored for tumorigenesis. Results are presented as mean values $\pm$ SD. $*P < 0.05$, using Student's t test.

SDH5, a Gene Required for Flavination of Succinate Dehydrogenase, Is Mutated in Paraganglioma

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Mammalian mitochondria contain about 1100 proteins, nearly 300 of which are uncharacterized. Given the well-established role of mitochondrial defects in human disease, functional characterization of these proteins may shed new light on disease mechanisms. Starting with yeast as a model system, we investigated an uncharacterized but highly conserved mitochondrial protein (named here Sdh5). Both yeast and human Sdh5 interact with the catalytic subunit of the succinate dehydrogenase (SDH) complex, a component of both the electron transport chain and the tricarboxylic acid cycle. Sdh5 is required for SDH-dependent respiration and for Sdh1 flavination (incorporation of the flavin adenine dinucleotide cofactor). Germline loss-of-function mutations in the human *SDH5* gene, located on chromosome 11q13.1, segregate with disease in a family with hereditary paraganglioma, a neuroendocrine tumor previously linked to mutations in genes encoding SDH subunits. Thus, a mitochondrial proteomics analysis in yeast has led to the discovery of a human tumor susceptibility gene.

Mitochondrial defects have been implicated in a variety of human disorders, including cancer (1, 2). Nearly one-third of the mammalian mitochondrial proteome is currently uncharacterized. Many of these uncharacterized proteins are evolutionarily conserved, a strong indication that they perform fundamentally important cellular functions (3). We initiated a project to determine the function of one of these proteins, named here Sdh5, using yeast as the primary model system. The Sdh5 protein family is highly conserved in eukaryotes and in some prokaryotic species, including *Rickettsia*, which is related to the bacterium that became the ancestral mitochondrion (fig. S1) (4, 5).

The mitochondrial localization of yeast Sdh5 (originally named EMI5/YOL071W) was suggested by proteomics studies (6), and we confirmed this by fluorescence microscopy of a strain expressing Sdh5–green fluorescent protein and subcellular fractionation of a strain expressing Sdh5–GFP (green fluorescent protein) (figs. S2 and S3). Both forms of tagged Sdh5 were expressed from the native *SDH5*

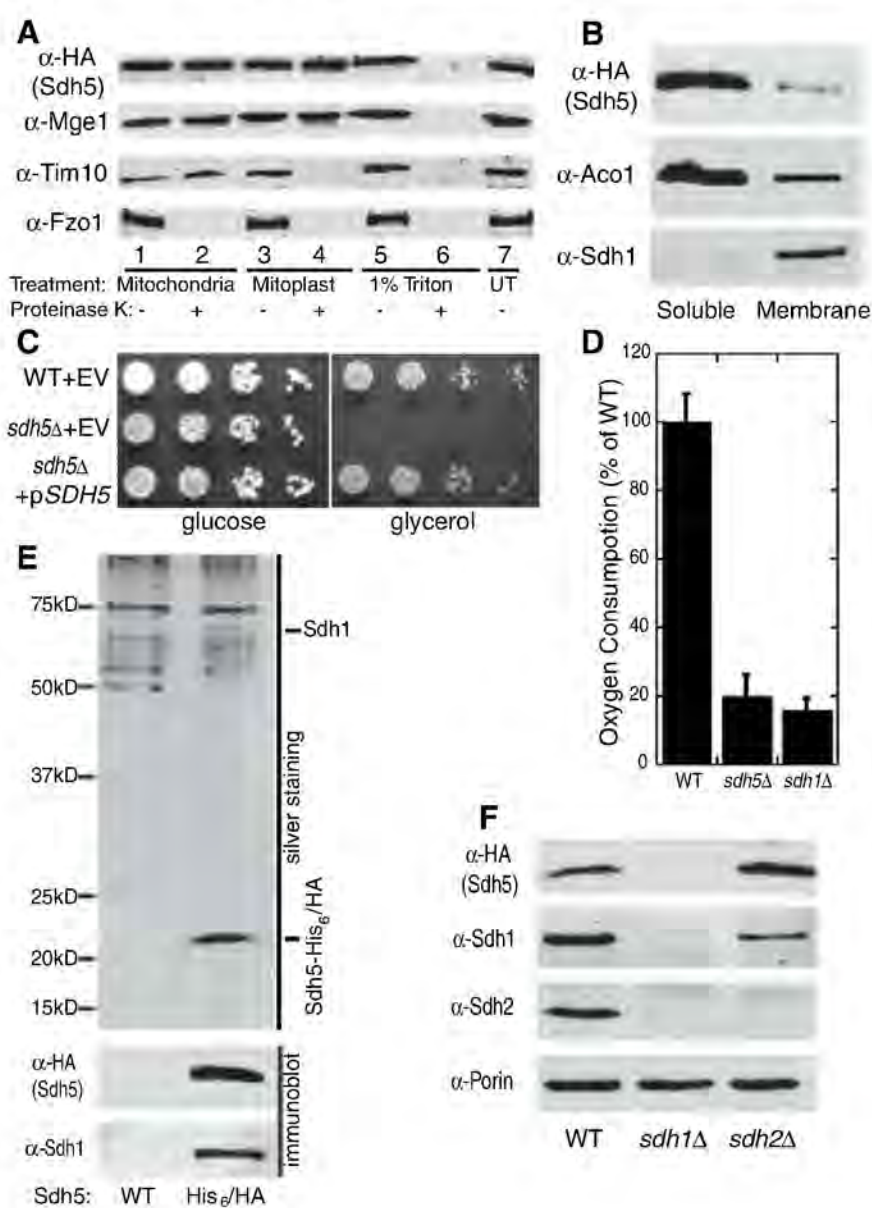
promoter and were fully functional. We further showed that Sdh5 resides in the mitochondrial matrix (Fig. 1A) and is predominantly soluble (Fig. 1B).

Respiratory-deficient mutants of *S. cerevisiae* are viable on fermentable carbon sources such as glucose, but are inviable on nonfermentable carbon

sources such as glycerol. We found that a yeast strain with a deletion of *SDH5* (*sdh5Δ*) grew normally on glucose medium but failed to grow on glycerol medium, a phenotype that was rescued by an *SDH5*-expressing plasmid (Fig. 1C). The *sdh5Δ* strain also showed impaired oxygen consumption, similar to the respiratory-deficient *sdh1Δ* strain (7) (Fig. 1D), as well as the respiration-related phenotypes of H₂O₂ hypersensitivity and decreased chronological life-span (figs. S4 and S5). The *sdh5Δ* respiratory deficiency was not due to defective mitochondrial DNA (mtDNA), because the *sdh5Δ* strain complemented the glycerol growth defect of a *rho*⁰ (mtDNA-deficient) strain in mating tests (fig. S6). Thus, the *sdh5Δ* strain is respiratory-deficient, despite having a functional mitochondrial genome.

Silver staining of the final eluates from tandem affinity purification of Sdh5–His₆/HA revealed two proteins specific for tagged Sdh5 as compared to the wild type, including Sdh5 itself at ~22 kD (Fig. 1E). The second migrated at ~70 kD and was identified by mass spectrometry as Sdh1, the catalytic subunit of the succinate dehydrogenase (SDH) complex. The presence of both Sdh5 and Sdh1 in the final eluate was confirmed by immunoblot (Fig. 1E). The SDH complex is a component of both the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC). In the latter, the SDH complex is known as complex II. It is a highly conserved heterotetramer, with Sdh1

Fig. 1. Sdh5 is required for respiration and interacts with Sdh1. (A) Mitochondria, mitoplasts generated by hypotonic swelling, and 1% Triton X-100-solubilized mitochondria from a strain expressing Sdh5–HA were treated with (+) or without (–) proteinase K and analyzed by immunoblotting with an untreated mitochondria control (UT). Mge1, Tim10, and Fzo1 are matrix, intermembrane space, and outer membrane proteins, respectively. (B) Soluble and membrane fractions of purified mitochondria (4) as in (A) were immunoblotted. Aco1, soluble matrix protein; Sdh1, membrane-associated matrix protein. (C) Serial dilutions of WT and *sdh5Δ* strains containing empty vector (EV) or a plasmid expressing Sdh5–HA were spotted on glucose or glycerol medium and grown at 30°C for 2 or 3 days, respectively. (D) Oxygen consumption in WT, *sdh5Δ*, and *sdh1Δ* strains grown to mid-log phase in raffinose media (±SD, *n* = 3 biological replicates). Similar results were obtained in glucose medium. (E) Tandem purification eluates (4) from a WT strain and a strain expressing Sdh5–His₆/HA were resolved with SDS-PAGE and visualized by silver staining (top panel) or immunoblot with antibodies to Sdh1 and HA (lower panels). (F) Immunoblot of purified mitochondria from WT, *sdh1Δ*, or *sdh2Δ* strains expressing Sdh5–HA. Porin, mitochondrial loading control.



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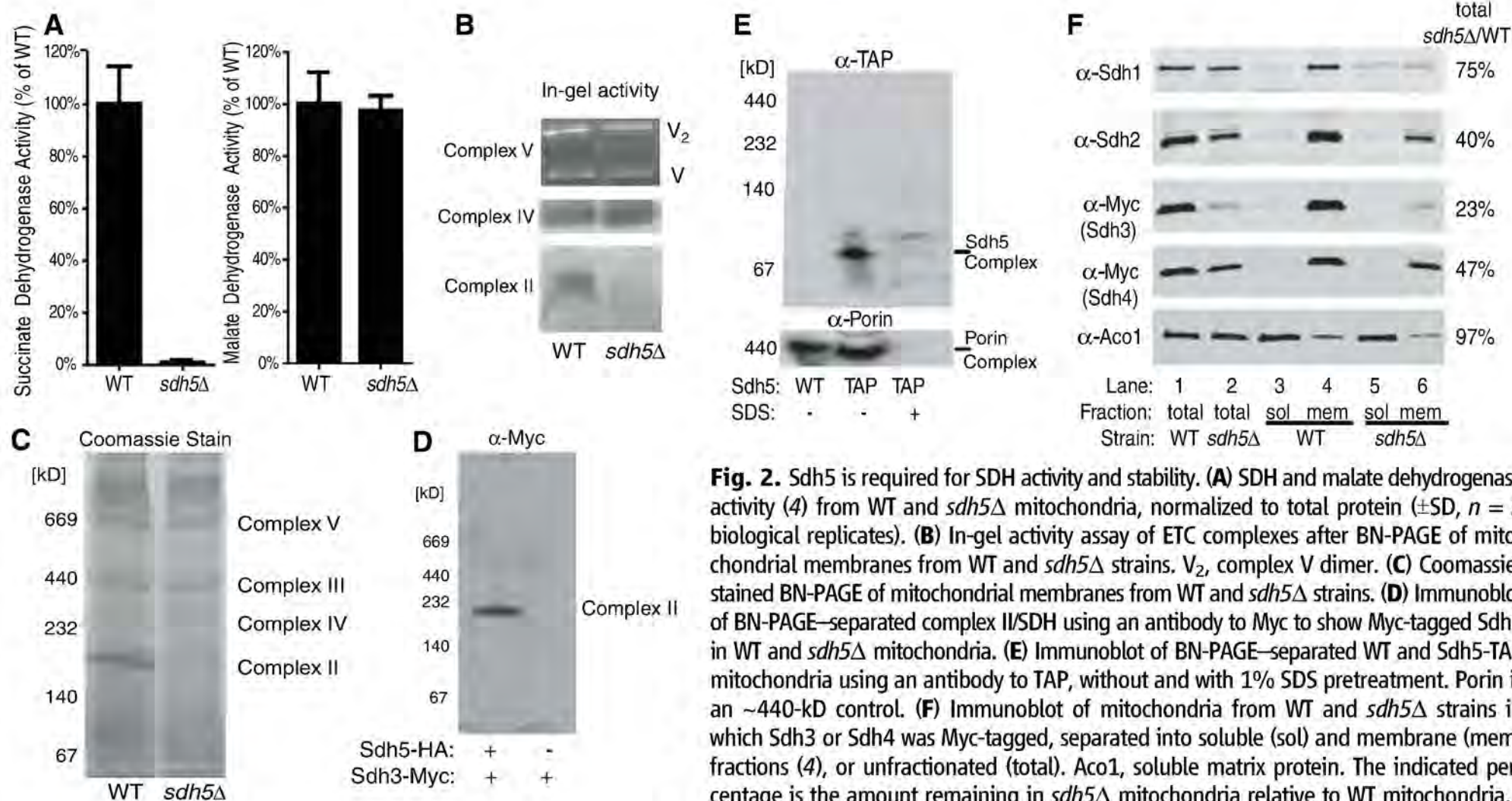
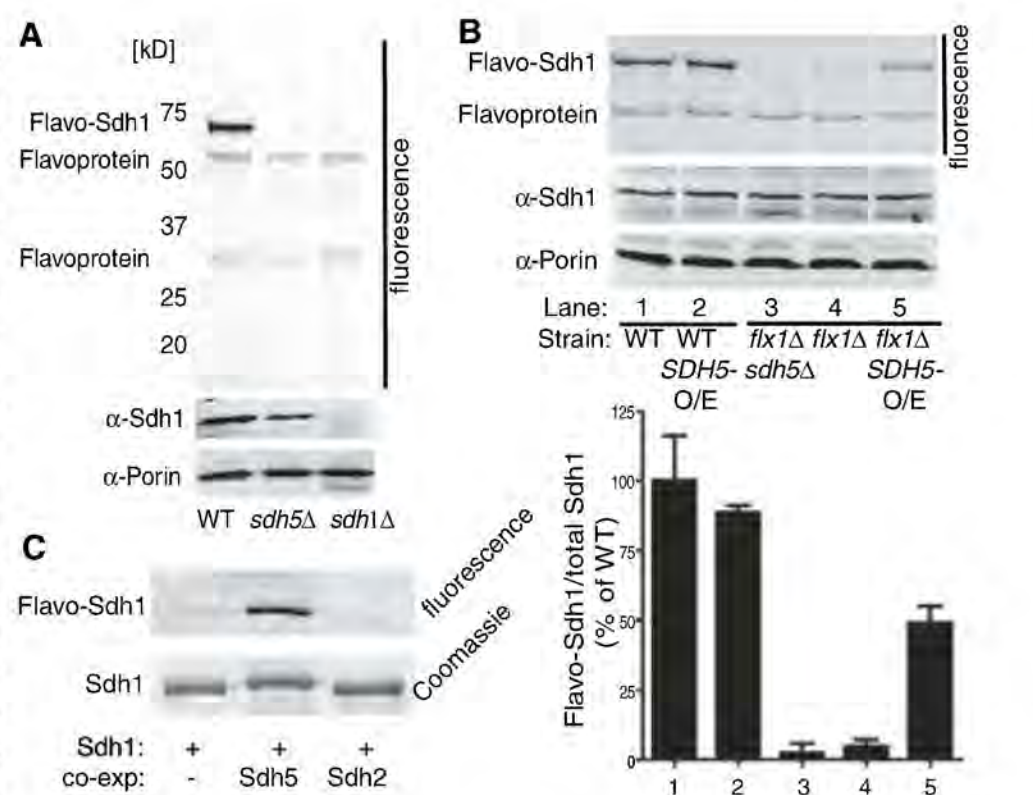


Fig. 2. Sdh5 is required for SDH activity and stability. (A) SDH and malate dehydrogenase activity (4) from WT and *sdh5Δ* mitochondria, normalized to total protein ($\pm$ SD, $n = 3$ biological replicates). (B) In-gel activity assay of ETC complexes after BN-PAGE of mitochondrial membranes from WT and *sdh5Δ* strains. V₂, complex V dimer. (C) Coomassie-stained BN-PAGE of mitochondrial membranes from WT and *sdh5Δ* strains. (D) Immunoblot of BN-PAGE-separated complex II/SDH using an antibody to Myc to show Myc-tagged Sdh3 in WT and *sdh5Δ* mitochondria. (E) Immunoblot of BN-PAGE-separated WT and Sdh5-TAP mitochondria using an antibody to TAP, without and with 1% SDS pretreatment. Porin is an ~440-kD control. (F) Immunoblot of mitochondria from WT and *sdh5Δ* strains in which Sdh3 or Sdh4 was Myc-tagged, separated into soluble (sol) and membrane (mem) fractions (4), or unfractionated (total). Aco1, soluble matrix protein. The indicated percentage is the amount remaining in *sdh5Δ* mitochondria relative to WT mitochondria.

and Sdh2 as catalytic subunits anchored to the mitochondrial inner membrane by Sdh3 and Sdh4 (fig. S12) (8). Both *sdh1Δ* and *sdh5Δ* mutants were respiratory-deficient and failed to grow on glycerol medium, but grew weakly with ethanol as the carbon source (Fig. 1D and fig. S7) and exhibited acetate hyperexcretion, a phenotype shared by only four other TCA cycle mutants (9). The importance of the Sdh1-Sdh5 interaction was confirmed by the observation that Sdh5 (like Sdh2) is completely degraded in the *sdh1Δ* strain (Fig. 1F). In contrast, loss of *SDH2* led to an increase in the Sdh5 protein level (Fig. 1F), presumably due to enhanced Sdh1/Sdh5 complex formation in the absence of Sdh2, the major Sdh1 partner.

The Sdh1-Sdh5 interaction is likely to be functionally important because the *sdh5Δ* mutant lacks SDH activity (Fig. 2A), as previously observed for the *sdh1Δ* mutant (10). The activity of malate dehydrogenase, another TCA cycle enzyme, was not affected by *SDH5* deletion (Fig. 2A). Because SDH is complex II in the ETC, we performed in-gel activity staining of ETC complexes after separation by blue native-polyacrylamide gel electrophoresis (BN-PAGE). As shown in Fig. 2B, complex II/SDH activity was absent in the *sdh5Δ* mutant, whereas the activities of complexes IV and V were normal. We then examined ETC complex assembly and stability by Coomassie blue staining after BN-PAGE and found that complex II/SDH was specifically absent in *sdh5Δ* mitochondria (Fig. 2C). The loss of complex II/SDH in the *sdh5Δ* mutant was confirmed by anti-Myc immunoblot after BN-PAGE of wild-type (WT) and *sdh5Δ* strains expressing Sdh3-Myc (Fig. 2D). Sdh5 is not a stable component of complex II/SDH because it migrates in a ~90-kD SDS-sensitive com-

Fig. 3. Sdh5 is necessary and sufficient for Sdh1 flavination. (A) WT, *sdh5Δ*, and *sdh1Δ* mitochondria were resolved by SDS-PAGE and imaged (4) for covalent FAD (top panel) or immunoblotted (lower panels). (B) Fluorescence gel image (top panel) and immunoblot (lower panels) as in (A), with whole-cell extract from WT or *flx1Δ* *sdh5Δ* strains containing EV, CEN plasmid *SDH5* (*flx1Δ*: ~1 copy per cell), or 2 μ plasmid *SDH5* (O/E: ~10 copies per cell). The bar graph shows normalized FAD fluorescence ($\pm$ SD, $n = 3$ biological replicates) (bottom panel). (C) His-tagged yeast Sdh1 was expressed alone or with Sdh5 or Sdh2 in *E. coli*, purified, and analyzed for FAD fluorescence as in (A) and by Coomassie blue staining.



plex during BN-PAGE (Fig. 2E), which is distinct from complex II (~200 kD). This ~90-kD complex is likely to be the Sdh5-Sdh1 (70-kD) heterodimer.

The levels of all four SDH subunits were significantly decreased in the *sdh5Δ* mutant, probably because of degradation in the absence of a stable SDH complex (Fig. 2F, lane 1 versus lane 2). The residual Sdh1 level was higher than that of the other subunits, but much of it was in the soluble fraction, unassociated with the SDH complex (Fig. 2F). These data suggest that the SDH complex assembles in the absence of Sdh5, but the complex is nonfunctional and Sdh1 is not stably bound. As a

result, the unstable complex is more susceptible to degradation and is disrupted by detergent extraction during the BN-PAGE procedure (the fractionation shown in Fig. 2F was detergent-free).

Multiple cofactors are required for activity of the SDH complex, including the flavin adenine dinucleotide (FAD) in Sdh1 (11). In other ETC complexes, cofactors are important for complex assembly and stability in addition to enzymatic activity (12). The strongly fluorescent FAD is covalently attached to Sdh1 and can be detected by in-gel fluorimetry after SDS-PAGE (13). Deletion of *SDH5* caused a complete loss of FAD cofactor attachment (flavination)

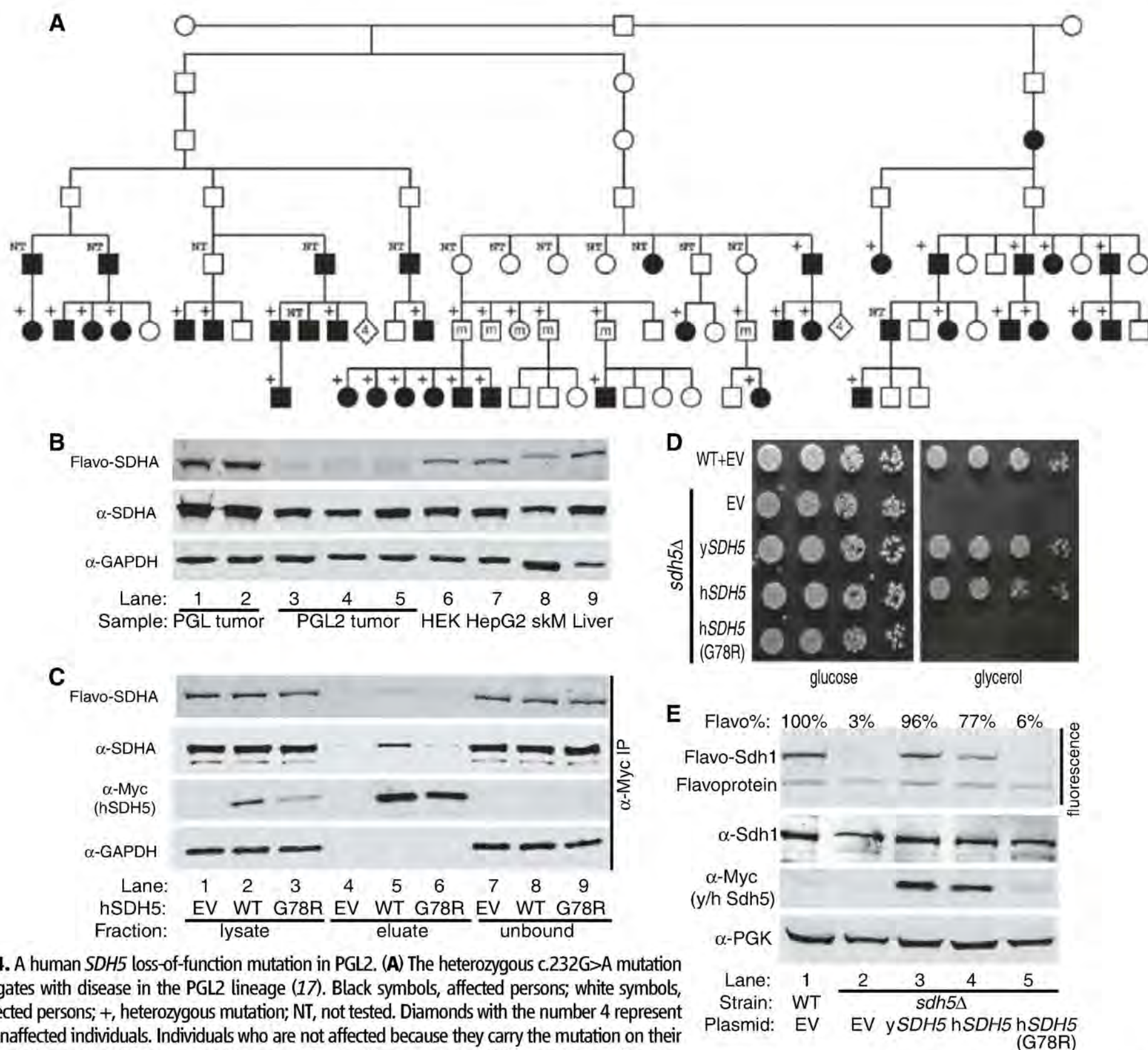


Fig. 4. A human *SDH5* loss-of-function mutation in PGL2. **(A)** The heterozygous c.232G>A mutation segregates with disease in the PGL2 lineage (17). Black symbols, affected persons; white symbols, unaffected persons; +, heterozygous mutation; NT, not tested. Diamonds with the number 4 represent four unaffected individuals. Individuals who are not affected because they carry the mutation on their maternal chromosome 11 are marked by an m. One healthy maternal mutation carrier and five non-affected paternal mutation carriers are not shown in the pedigree for privacy reasons. **(B)** Fluorescence gel image (top panel) and immunoblotting (lower panels) of samples from human tumors, cell lines, and mouse tissues. Lanes 1 and 2, sporadic PGL tumors; lanes 3 to 5, PGL2 tumors (hSDH5 G78R); lanes 6 and 7, HEK293 and HepG2 human cell lines; lanes 8 and 9, normal mouse skeletal muscle (skM) and liver. **(C)** Lysate from HEK293 cells containing EV or expressing WT or G78R hSDH5-Myc were immunoprecipitated with agarose beads conjugated with antibody to Myc. Lysate, eluate, and unbound fraction were FAD-imaged (top panel) and immunoblotted (lower three panels). **(D)** Serial dilutions of WT and *sdh5* Δ strains containing EV or plasmids expressing yeast Sdh5-Myc, WT human SDH5-Myc, or G78R hSDH5-Myc were spotted on glucose or glycerol medium and grown at 30°C for 2 or 3 days, respectively. **(E)** Fluorescence gel image (top panel) and immunoblotting of whole-cell extract from the five strains in (D) (lower panels). PGK, loading control. FAD fluorescence was normalized to Sdh1 protein level and expressed as a percentage relative to WT (Flavo%, $\pm$ SD, $n = 3$ biological replicates).

of Sdh1, although Sdh1 was still present (Fig. 3A). The flavination of two other mitochondrial flavo-proteins, visualized in the same gel, was unaffected by either *SDH5* or *SDH1* deletion (Fig. 3A).

Sdh5 overexpression in WT cells did not increase Sdh1 flavination (Fig. 3B, lane 1 versus lane 2), possibly due to flavination that was already stoichiometric. A significant effect of Sdh5 overexpression might require a state of reduced Sdh1 flavination as was observed in a strain with decreased mitochondrial FAD caused by deletion of the mitochondrial FAD transporter Flx1 (13, 14). The *flx1* Δ mutant displayed almost no Sdh1 flavination, but Sdh5 overexpression restored flavination to about 50% of WT levels (Fig. 3B,

lanes 4 and 5). As shown in Fig. 3C, FAD incorporation was almost undetectable in *Escherichia coli*-expressed Sdh1 but was increased dramatically when Sdh5, but not Sdh2, was coexpressed. These data demonstrate that Sdh5 is both necessary and sufficient for Sdh1 flavination.

The amino acid sequence of yeast Sdh5 is 44% identical (from residues 33 to 158 of 163) to its human ortholog C11orf79, which we rename here hSDH5 (fig. S1). We hypothesized that hSDH5 is required for flavination of SDHA (human Sdh1) and thus for SDH activity. Previous genetic studies have shown that mutations causing loss of SDH activity are responsible for hereditary forms of a rare neuroendocrine tumor called paraganglioma (PGL).

Of the four familial PGL syndromes (PGL1 to PGL4), PGL1, PGL3, and PGL4 have been associated with mutations in *SDHD*, *SDHC*, and *SDHB*, respectively (15). The gene for PGL2 (Online Mendelian Inheritance in Man number 601650) has not been identified, but it maps to chromosome 11q13.1, the genomic locus of the *hSDH5* gene (16).

We sequenced *hSDH5* in three affected individuals from different branches of the previously described Dutch PGL2 lineage (17). In all three individuals, we found a single nucleotide c.232G>A change in exon 2 (fig. S8), which causes a Gly⁷⁸ $\rightarrow$ Arg⁷⁸ (G78R) mutation in the most conserved region of the protein (fig. S1). None of 400 unaffected control individuals carried the c.232G>A

mutation. Within the affected lineage, the mutation was found to cosegregate with the disease haplotype in all 45 individuals who inherited this haplotype, and was not detected in 44 unaffected members without this haplotype. Thirty-three individuals with the mutation have developed the disease, but not seven individuals (2009 median age = 74 years) who inherited the mutation from their mother (Fig. 4A). This suggests an SDHD-like parent-of-origin-specific inheritance pattern. Only five individuals (2009 median age = 42 years) with a paternal mutation have not developed overt PGL. Because penetrance of the disease increases with age (17), these individuals may develop tumors in the future, or tumors may already be present but undetected.

SDHA flavination was decreased by ~95% in tumors from three patients with PGL2 (*hSDH5* G78R) in comparison with control tumors from two sporadic PGL patients or with cultured human embryonic kidney and hepatoma cells and mouse skeletal muscle and liver tissue (Fig. 4B). Mitochondrial localization of hSDH5 was not compromised by this mutation (fig. S9). As observed in yeast, SDHA coimmunoprecipitated with WT hSDH5-Myc. The G78R hSDH5 mutation, however, dramatically impaired the interaction of hSDH5 with SDHA and slightly decreased the level of hSDH5 protein (Fig. 4C). We conclude that the G78R mutation destabilizes hSDH5 and impairs its interaction with SDHA.

When expressed in yeast from the yeast *SDH5* promoter, WT hSDH5 complemented the *sdh5Δ* glycerol growth defect, but the G78R mutant had no effect (Fig. 4D). Overexpression of any of the individual SDH subunits or the proposed SDH chaperone Tcm62 (18) failed to complement the *sdh5Δ* mutant phenotype (fig. S10). Further, an extensive high-copy suppressor screen of the *sdh5Δ* glycerol growth defect using a yeast genomic library recovered many independent isolates of *SDH5* but failed to identify any other suppressing genes. Therefore, the specific rescue of the *sdh5Δ* glycerol growth defect by native-level expression of *hSDH5* is strong evidence of the conservation of Sdh5 function from yeast to humans. Furthermore, expression of hSDH5 in an *sdh5Δ* mutant strain increased the flavination of yeast Sdh1 to 77% of the WT level, but the G78R mutant had no effect (Fig. 4E). The lack of activity of the G78R *hSDH5* mutant in yeast is likely due to destabilization of the protein (Fig. 4E).

The mechanism of covalent FAD insertion into proteins is controversial. Although other covalent cofactors are inserted by specialized enzymes (19), an autocatalytic mechanism has been proposed for flavination (20). However, covalent FAD attachment to Sdh1 was shown to require at least one additional protein (21), which we hypothesize to be Sdh5. Whether Sdh5 participates in the chemistry of FAD attachment (enzymatic function) or simply maintains Sdh1 in a conformation that is susceptible to autocatalytic FAD attachment (chaperone function) is a question that remains to be addressed.

The identification of *hSDH5* as the *PGL2* gene has potential clinical implications. Approximately

70% of familial cases of head and neck PGL are due to germline mutations in *SDHB*, *SDHC*, or *SDHD* (22), and 10% of sporadic PGL cases are associated with mutations in *SDHB* or *SDHD* (23, 24). Mutations in *SDHB*, *SDHC*, and *SDHD* have also been detected in gastrointestinal stromal tumors (25), pheochromocytomas (24), renal cell carcinoma (26), and other diseases (27). Conceivably, *hSDH5* mutations may underlie disease development in patients who have tested negative for mutations in *SDHB*, *SDHC*, and *SDHD*. The inclusion of *hSDH5* will allow more comprehensive genetic testing, which is suggested for the clinical management of PGL, even for sporadic cases (28). The same is also true of the *SDHAF1* gene, which encodes a protein involved in SDH assembly and which was recently identified as a causative factor in infantile leukoencephalopathy (29). In summary, starting with a previously uncharacterized mitochondrial protein in yeast, we have shown through biochemical and genetic analyses that the protein plays a critical role in the biogenesis and function of a respiratory complex and that its mutational inactivation confers tumor susceptibility in humans.

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Supporting Online Material

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Eos Mediates Foxp3-Dependent Gene Silencing in CD4⁺ Regulatory T Cells

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CD4⁺ regulatory T cells (T_{regs}) maintain immunological self-tolerance and immune homeostasis by suppressing aberrant or excessive immune responses. The core genetic program of T_{regs} and their ability to suppress pathologic immune responses depends on the transcription factor Foxp3. Despite progress in understanding mechanisms of Foxp3-dependent gene activation, the molecular mechanism of Foxp3-dependent gene repression remains largely unknown. We identified Eos, a zinc-finger transcription factor of the Ikaros family, as a critical mediator of Foxp3-dependent gene silencing in T_{regs}. Eos interacts directly with Foxp3 and induces chromatin modifications that result in gene silencing in T_{regs}. Silencing of Eos in T_{regs} abrogates their ability to suppress immune responses and endows them with partial effector function, thus demonstrating the critical role that Eos plays in T_{reg} programming.

Naturally occurring CD4⁺CD25⁺ regulatory T cells (T_{regs}), which specifically express the transcription factor Foxp3,

are essential for the suppression of pathological immune responses to both self and foreign antigens (1–3). Although it has been shown that

Foxp3 interacts with a number of transcription and chromatin-modifying regulators (4–7), the molecular mechanism by which Foxp3 mediates selective gene silencing in T_{regs} is still largely unknown. For example, the silencing of cytokine genes such as those that express interleukin-2 (IL-2) and interferon- γ (IFN- γ) is critical in assuring that T_{regs} suppress rather than facilitate immune responses.

To gain insight into the molecular mechanisms of T_{reg} function, we conducted comparative gene chip analysis on antigen-specific $CD4^+$ T cells under tolerizing versus activating conditions (8). One of the genes that exhibited substantially higher expression in tolerizing/ T_{reg} conditions relative to activating conditions was *Eos*, which expresses a zinc-finger transcription factor that belongs to the Ikaros family. We confirmed the result by real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) and Western blot analysis. *Eos* was highly expressed in the $CD4^+CD25^+$ and $CD4^+Foxp3^+$ T_{reg} population (Fig. 1, A and B).

Given that both *Eos* and Foxp3 are highly expressed in T_{regs} and that *Eos* exhibits transcrip-

tional repression activity in certain tumor cell lines (9), we speculated that *Eos* might interact with Foxp3 and mediate its gene-silencing activity. To test this hypothesis, we carried out coimmunoprecipitation between *Eos* and Foxp3 ectopically expressed in Jurkat T cells (a transformed human T cell line), which lack endogenous Foxp3. *Eos* was found to coimmunoprecipitate with Foxp3 (fig. S1A). Endogenous Foxp3 and *Eos* also coimmunoprecipitated in lysates prepared from natural T_{regs} (Fig. 1, C and D). Similar experiments in cell lines and natural T_{regs} revealed that two other Ikaros family members (Ikaros and Helios) did not interact with Foxp3 (figs. S1, B and C, and S2). To verify that the interaction between *Eos* and Foxp3 is direct, we used affinity-purified recombinant glutathione *S*-transferase (GST)–*Eos* fusion protein (fig. S1D) to bind [35 S]-labeled Foxp3 that was produced by in vitro transcription and translation. GST–*Eos*, but not GST, bound to [35 S]-Foxp3, which suggests a direct interaction between the two proteins in vitro (Fig. 1E). Next, we determined the minimal *Eos*-interacting domain in Foxp3 using coimmunoprecipitation. Human embryonic kidney (HEK) 293T cells were cotransfected with constructs encoding various truncated forms of *Eos* and Foxp3. This approach led to the identification of a 51-residue (amino acids 148 to 198) fragment of Foxp3 that is necessary and sufficient to bind to the C-terminal region (amino acids 281 to 586) of *Eos* (fig. S3).

In T_{regs} , the IL-2 locus is silenced, and Foxp3 has been shown to be responsible for the suppression of IL-2 expression in both T cell lines

and primary T cells (4). We thus investigated whether *Eos* was involved in IL-2 suppression by Foxp3 in T cells by using small interfering RNA (siRNA) to inhibit (knockdown) *Eos* (si-*Eos*) in primary $CD4^+$ T cells (10) (Fig. 2A) that were then transduced with a bicistronic retroviral vector expressing full-length Foxp3 or a deletion of the 51-amino acid fragment that is required for *Eos* interaction (Δ Foxp3). As expected, the expression of the full-length Foxp3 almost completely blocked IL-2 production (Fig. 2B), and *Eos* protein expression remained unchanged upon overexpression of Foxp3 (fig. S4). Δ Foxp3 completely abrogated the ability of Foxp3 to repress IL-2 production. Knockdown of *Eos* reversed Foxp3-mediated suppression of IL-2 production (Fig. 2B), which could be rescued by retransduction with a retroviral vector expressing *Eos* with one base pair mutation at the siRNA-targeted region. Together, these results indicate that Foxp3-mediated IL-2 gene suppression requires *Eos* and the *Eos*-interacting domain of Foxp3.

C-terminal binding protein-1 (CtBP1) has been implicated as a key repressor of *Eos*-mediated gene expression (9, 11–13); thus, we hypothesized that *Eos* might serve as a co-repressor in T_{regs} to recruit CtBP1 to Foxp3. To test this hypothesis, we determined the association between *Eos* and CtBP1 and assessed IL-2 promoter epigenetic status on *Eos* knockdown in T_{regs} . *Eos* indeed interacted with CtBP1 in T_{regs} (Fig. 2C). Moreover, both *Eos* and CtBP1 coimmunoprecipitated with Foxp3, whereas knockdown of either *Eos* or Δ Foxp3 [which is still capable of associating with NFAT (nuclear factor

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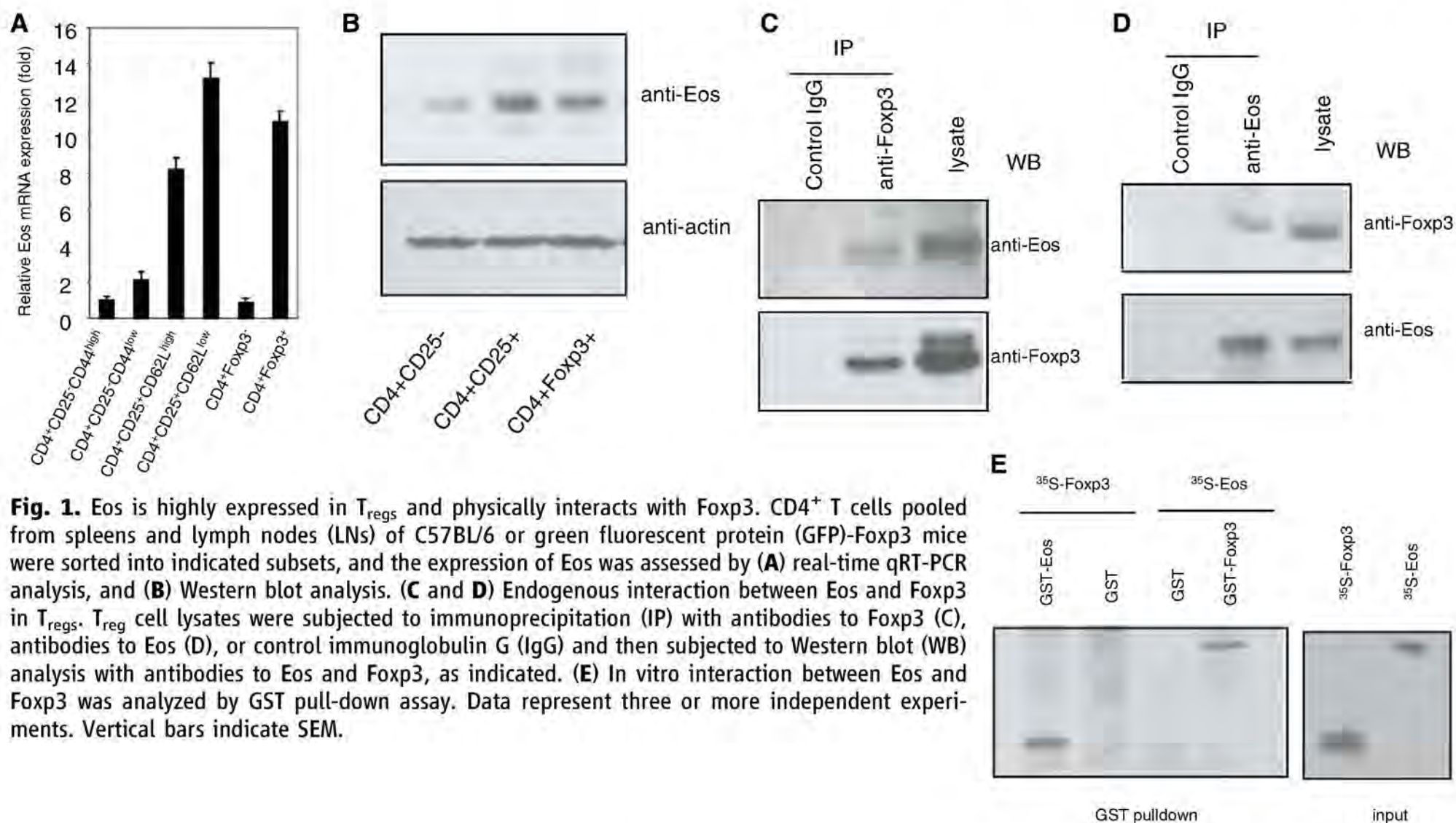


Fig. 1. *Eos* is highly expressed in T_{regs} and physically interacts with Foxp3. $CD4^+$ T cells pooled from spleens and lymph nodes (LNs) of C57BL/6 or green fluorescent protein (GFP)-Foxp3 mice were sorted into indicated subsets, and the expression of *Eos* was assessed by (A) real-time qRT-PCR analysis, and (B) Western blot analysis. (C and D) Endogenous interaction between *Eos* and Foxp3 in T_{regs} . T_{reg} cell lysates were subjected to immunoprecipitation (IP) with antibodies to Foxp3 (C), antibodies to *Eos* (D), or control immunoglobulin G (IgG) and then subjected to Western blot (WB) analysis with antibodies to *Eos* and Foxp3, as indicated. (E) In vitro interaction between *Eos* and Foxp3 was analyzed by GST pull-down assay. Data represent three or more independent experiments. Vertical bars indicate SEM.

of activated T cells) or AML1/Runx1 (acute myeloid leukemia 1/runt related transcription factor 1) while not binding Eos (fig. S5)] abrogated the incorporation of CtBP1 into Foxp3-suppressive complexes (Fig. 2D). These data suggest that CtBP1 is part of the Foxp3-Eos suppression complex.

It has been reported that CtBP1 suppresses transcription by affecting histone methylation and acetylation (11). To determine whether this was the case in T_{reg} , we applied chromatin immunoprecipitation (ChIP) analysis to detect the histone modifications at the IL-2 promoter region in T_{reg} by examining histone 3 lysine 4 (H3K4) and lysine 9 (H3K9) methylation, as well as histone 3 and histone 4 acetylation (H3Ac, H4Ac). In vitro-primed effector CD4⁺ cells, which transcribe the IL-2 gene more rapidly than naïve cells do after activation (14), exhibited a 10- to 40-fold increase in histone trimethylation (Me3H3K4) and acetylation (H3Ac and H4Ac), but a concurrent decrease in histone methylation (MeH3K9) at the IL-2 promoter (Fig. 2E). In contrast, T_{reg} exhibited little or no change in histone methylation and acetylation at the IL-2 promoter compared to that of naïve T cells. Upon knockdown of Eos,

histone trimethylation (Me3H3K4) and acetylation (H3Ac and H4Ac) were significantly increased at the IL-2 promoter (Fig. 2E) but not at the CD3 ϵ promoter that does not contain a Foxp3 binding site (fig. S6). We attribute the changes in epigenetic modifications of histones at the IL-2 promoter to abrogation of the Foxp3 interaction with CtBP1 in si-Eos T_{reg} (Fig. 2D), because Foxp3 itself still binds to the IL-2 promoter even after si-Eos knockdown (fig. S7). CtBP1 associates with a co-repressor complex including EuHMT (G9a), which is responsible for H3K9 methylation in other cell types (15, 16). We thus used RNA interference (RNAi) to determine whether CtBP1 is associated with the methylation of H3K9 at the IL-2 promoter in T_{reg} . Upon knockdown of CtBP1 by RNAi (fig. S8A), we observed a marked decrease in CtBP1 and EuHMT occupancy at the IL-2 promoter, whereas there was no significant change to Foxp3-Eos occupancy. CtBP1 knockdown was accompanied by a significant decrease in H3K9 methylation (MeK9, fig. S8B). In addition, we observed that histone H3 and H4 acetylation was enhanced upon si-CtBP1 treatment (Fig. 2F). At the functional level, knockdown of CtBP1 in T_{reg} abrogated

their suppressive activity (fig. S9). Similar changes in epigenetic histone modifications were also observed at the IFN- γ promoter in T_{reg} (fig. S10).

Another epigenetic modification that regulates IL-2 gene expression is methylation of genomic DNA at CpG dinucleotides in the promoter. We thus determined whether knockdown of Eos influenced the extent of DNA methylation at the IL-2 locus. Given the documented negative correlation between methylation status of the -68 CpG dinucleotide in the IL-2 promoter region and IL-2 transcription (17, 18), we examined the DNA methylation status of the CpG islands flanking the -68 region within the IL-2 promoter using a methylation-sensitive PCR assay in T_{reg} . Knockdown of Eos or CtBP1 led to demethylated CpG dinucleotides at the IL-2 promoter upon CD3 and CD28 treatment (Fig. 2G). Taken together, these findings lend further support to the notion that Eos or CtBP represses the IL-2 promoter through epigenetic modification mechanisms in T_{reg} .

We next sought to determine whether Eos plays a broader role in Foxp3-mediated T_{reg} gene silencing without affecting gene activation. Using the Foxp3-dependent gene set from natural

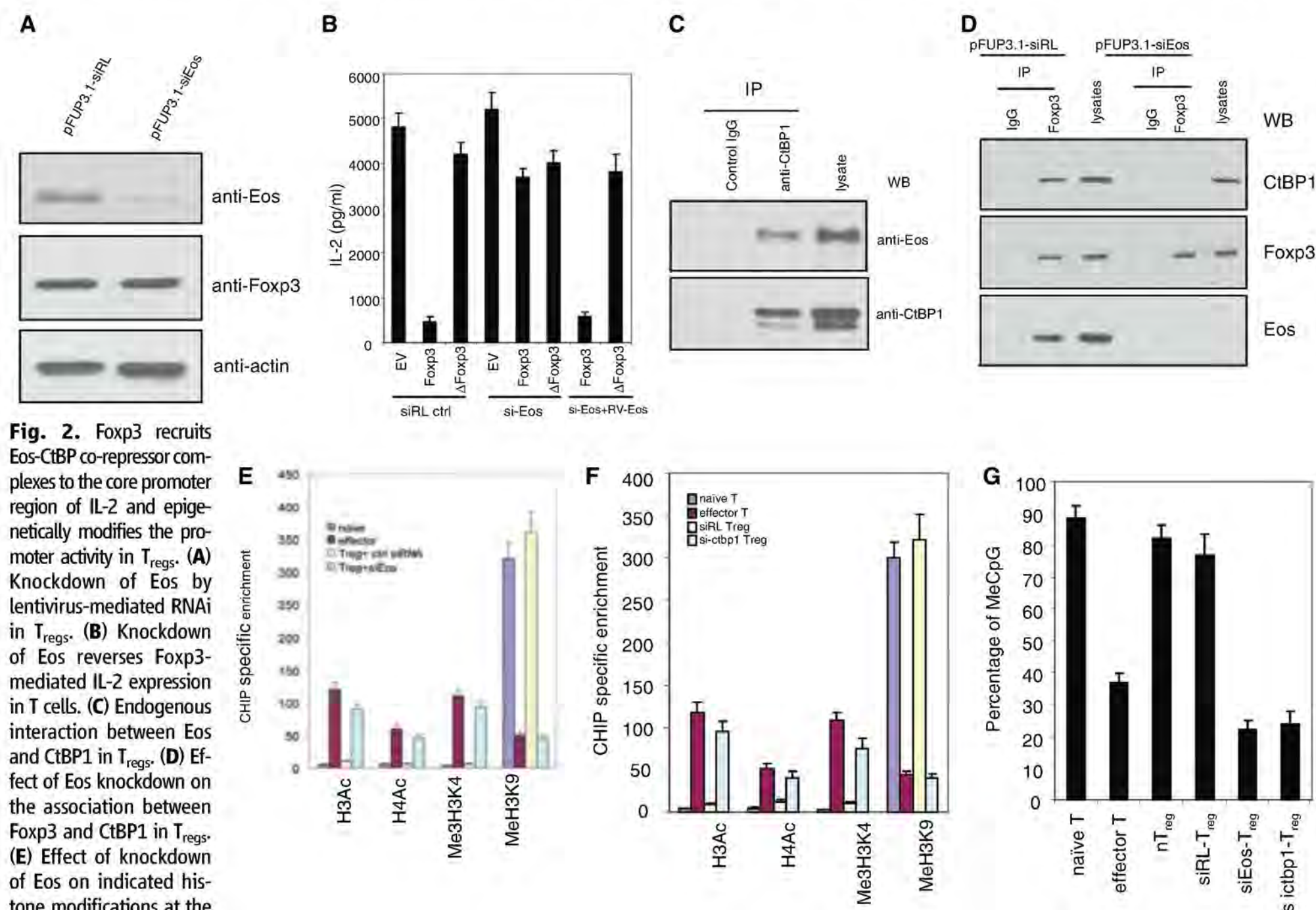


Fig. 2. Foxp3 recruits Eos-CtBP co-repressor complexes to the core promoter region of IL-2 and epigenetically modifies the promoter activity in T_{reg} . (A) Knockdown of Eos by lentivirus-mediated RNAi in T_{reg} . (B) Knockdown of Eos reverses Foxp3-mediated IL-2 expression in T cells. (C) Endogenous interaction between Eos and CtBP1 in T_{reg} . (D) Effect of Eos knockdown on the association between Foxp3 and CtBP1 in T_{reg} . (E) Effect of knockdown of Eos on indicated histone modifications at the Foxp3-binding region of the IL-2 promoter in T_{reg} . (F) Effect of knocking down CtBP1 on histone H3 and H4 modifications in the Foxp3-binding region at the IL-2 promoter in T_{reg} . (G) Effect of Eos and CtBP1 knockdown on the DNA

methylation status at the core region of the IL-2 promoter in T_{reg} . All primers for the ChIP analysis are listed in table S2. Data represent three or more independent experiments. Error bars indicate SEM.

T_{regs} defined by Rudensky and colleagues (19) as a basis for comparison, we analyzed changes in global gene expression patterns from Eos-siRNA versus control *Renilla* luciferase (RL)-siRNA-transduced T_{regs} . The majority (>70%) of the si-Eos-affected genes were Foxp3-dependent (fig. S11). Among them, 90% of the genes known to be suppressed by Foxp3 were no longer down-regulated upon Eos knockdown in T_{regs} (Fig. 3A). In marked contrast, >95% of known Foxp3-dependent up-regulated genes (19) were unaltered by Eos knockdown (Fig. 3B), a subset of which was further verified by qRT-PCR (Fig. 3C) and cell-surface staining (table S1).

The selective effect of Eos knockdown on Foxp3-repressed genes prompted us to study the specific role of Eos in T_{reg} function. Sorted T_{regs} , which were transduced with the same si-Eos lentivirus as above, were subjected to an in vitro suppression assay based on carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution

(20). Although both fresh wild-type T_{regs} and si-RL-transduced T_{regs} could suppress the proliferation of naïve T cells, T_{regs} with Eos knockdown were not suppressive but could be rescued by a functional human Eos construct (hEos) lacking the siRNA-targeted region (fig. S12). To further confirm that Eos is involved specifically in Foxp3-mediated suppression (21), we used recombination-activating gene (RAG)-deficient (*Rag2*^{-/-}) DO11.10 CD4⁺ T cells, which express ovalbumin (OVA) peptide-specific transgenic T cell receptors. Transgenic CD4⁺ T cells transduced with MIGR1-Foxp3-RFP suppressed the proliferation of freshly prepared nontransduced transgenic T cells upon stimulation with specific OVA peptides, whereas those transduced with the control MIGR1-RFP or MIGR1-ΔFoxp3-RFP mutant (148– to 198–amino acid deletion that is unable to interact with Eos) did not (Fig. 4A). siRNA knockdown of Eos reduced the suppressive activity of Foxp3-expressing antigen-specific

CD4⁺ T cells, which could be rescued by retransduction with RV-Eos or chimeric construct ΔFoxp3/hEos (Fig. 4A). Moreover, si-Eos treatment did not block suppressive activity conferred by a chimeric Foxp3/hEos gene (Fig. 4A). Foxp3 protein abundance remained unchanged upon si-Eos treatment (fig. S13). Together, these results suggest that Eos is required for Foxp3-mediated suppressive activity of T_{regs} .

Finally, we determined the role of Eos in the function of T_{regs} in an in vivo colitis model, where severe T helper type 1 (T_H1)-mediated inflammation within the colon is induced after the transfer of naïve CD4⁺CD25⁻CD62L^{high} T cells in the absence of T_{regs} into *Rag2*^{-/-} recipient mice (22). The cotransfer of CD4⁺CD25⁺ T_{regs} is sufficient to prevent the development of disease (23). Disease progression was monitored by weight loss, and colitis was assessed by histological analysis of colon tissue (24). Wild-type or si-RL-modified T_{regs} effectively suppressed the development of disease as judged by both body weight loss (Fig. 4B) and histological analysis of tissue isolated from the colon (Fig. 4, C and D). Transfer of si-Eos T_{regs} in the presence of CD4⁺CD25⁻CD62L^{high} T effector (T_{eff}) cells, however, failed to prevent the disease development (Fig. 4, B to D). In vivo tracking of transferred cells demonstrated that the colitis that developed in mice receiving CD4⁺CD25⁻CD62L^{high} T cells and si-Eos-transduced T_{regs} was not due to impaired accumulation or homing of Foxp3 T_{regs} to the mesenteric lymph nodes or spleen (Fig. 4, E to H). Given that Eos knockdown de-represses effector cytokine genes in T_{regs} , we asked whether T_{regs} developed effector function in the absence of Eos. Indeed, transfer of a higher dose of pure si-Eos T_{regs} (1×10^6) into *Rag2*^{-/-} mice produced colitis (figs. S14 to S16), which was less severe than that caused by transfer of the same number of T_{eff} cells. At a lower dose of transferred cells (2×10^5), T_{eff} cells still induced colitis, whereas si-Eos T_{regs} did not (fig. S15). Thus, Eos knockdown alone partially converts T_{reg} into functional T_{eff} cells, consistent with our finding that immune-suppressive functions associated with Foxp3-activated genes in T_{regs} are unaffected by Eos knockdown.

Collectively, our findings demonstrate that Foxp3 suppresses gene expression through its interaction with Eos and define Eos as a key missing link in this process. These results also raise the possibility that the interaction between Foxp3 and Eos, along with its associated co-repressor complexes, might be a potential therapeutic target for controlling physiological and pathological immune responses. This may be particularly true in the setting of cancer and chronic infections, where excessive T_{reg} activity seems to play an important role. Further dissection of the subset of genes silenced by Eos-Foxp3 complexes will likely lead to the identification of downstream molecules involved in the regulation of T_{reg} function.



Fig. 3. si-Eos could reverse most Foxp3-mediated suppression genes in T_{regs} . Shown are the genes of up-regulated (A) and down-regulated (B) transcripts in si-Eos versus si-RL-transduced T_{regs} . Naïve T cell and naïve T_{reg} gene expression profiling was carried out in parallel. (C) Knockdown of Eos enhances IL-2 and IFN- γ gene expression but does not affect Foxp3, CTLA-4, or GITR gene expression in T_{regs} . si-Eos and si-RL T_{regs} were sorted out and subjected to qRT-PCR assay with the indicated gene-specific primers. The results shown are the means $\pm$ SEM of three independent experiments.

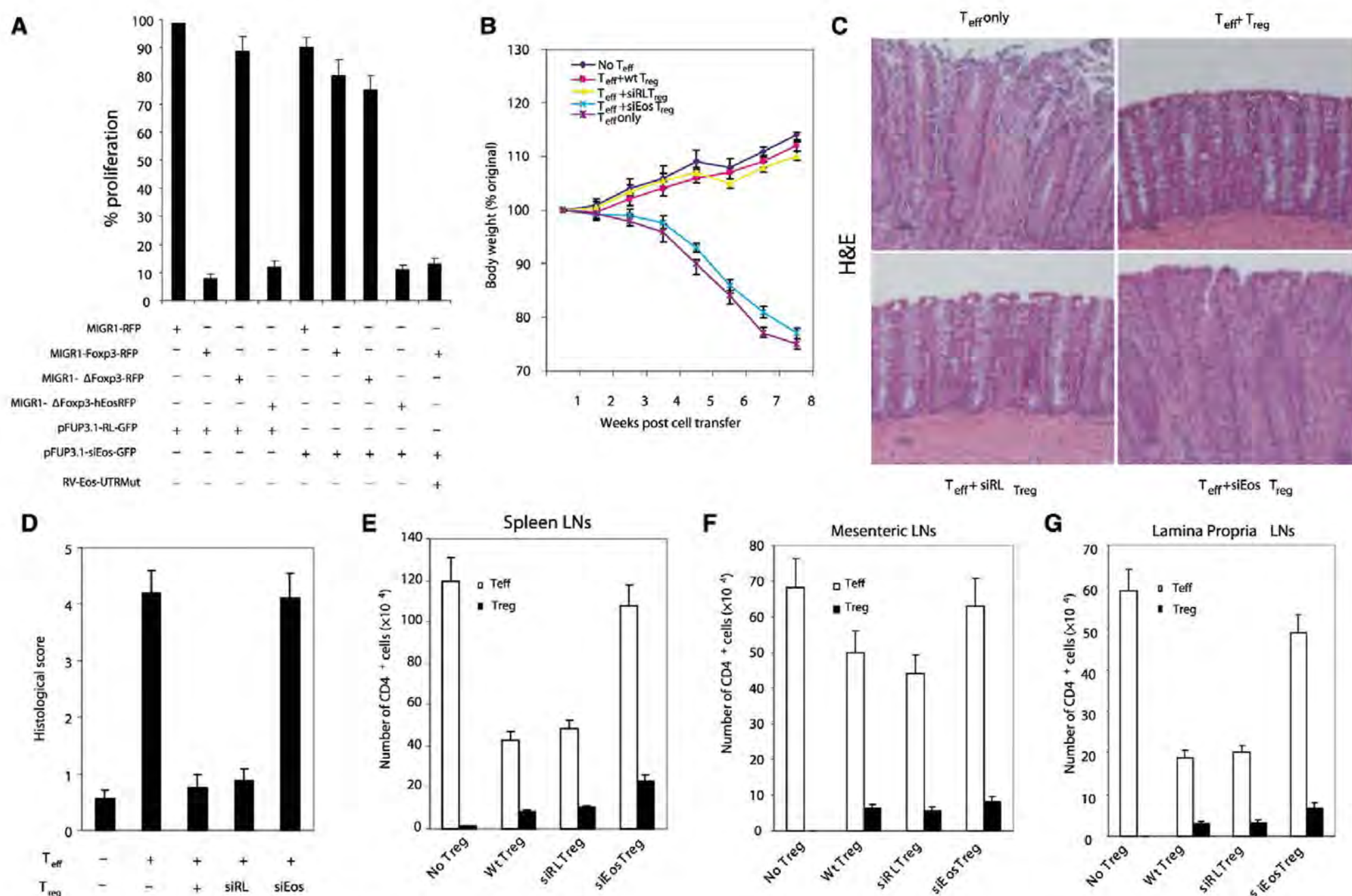


Fig. 4. Effects of knockdown of Eos on the suppressive activity of T_{regs} in vitro and in vivo. **(A)** Knockdown of Eos by siRNA reduces the suppressive activity of Foxp3-transduced naïve T cells. The results are representative of one of three independent experiments. **(B)** Percentage weight change after transfer of the indicated cells demonstrates that cotransfer of si-Eos-transduced T_{regs} cannot suppress weight loss induced by transfer of CD4⁺CD25⁺CD62L^{high} T_{eff} cells into *Rag2*^{-/-} mice. Nine mice were used in each group, and the results shown are the means ± SD of three independent experiments. **(C)** Representative photomicrography of the distal colon of *Rag2*^{-/-} mice after T cell transfer. **(D)** Colonic histology scores of experimental mice. **(E to G)** Analysis of spleen (E), mesenteric LNs (F), and lamina propria LN (G) T_{eff} (CD4⁺Foxp3⁻) and T_{reg} (CD4⁺Foxp3⁺) cell numbers. Eight to 10 mice were used in each group, and the results shown are the means ± SD of three

independent experiments. **(H)** Immunohistochemistry (IHC) staining of CD3⁺ or Foxp3⁺ T cells in colon tissue. Hematoxylin serves as a counterstain (magnification 20×). The IHC analysis was performed on five mice per group.

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Materials and Methods
Figs. S1 to S16
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MOLECULAR BIOENGINEERING OF BIOMASS CONVERSION

We seek an individual with experience in metabolic engineering of industrially significant microbes (e.g. yeast, fiber-degrading bacteria, clostridia) for production of biofuels from biomass feedstocks. The ideal candidate will have expertise in modern molecular biology techniques, whole genome shuffling, DNA microarrays, proteomic and metabolic tools, transposon mutagenesis and high-throughput screening methods, to maximize the production of bioproducts and biofuels. (Hans Blaschek, Theme Leader)

BIOCOMPLEXITY

We seek a quantitative scientist with interests in evolution, systems biology, and ecosystem dynamics, and expertise in statistical physics as applied to biology. The successful candidate will join a multi-disciplinary group exploring collective effects in biology. Projects include the evolution of translation, the role of horizontal gene transfer in communities of microbes and phages, and the systems biology of cells and ecosystems. (Nigel Goldenfeld, Theme Leader)

REGENERATIVE BIOLOGY & TISSUE ENGINEERING

The Fellow will be involved in one or more of our multidisciplinary projects related to regenerative biology and harnessing the potential of adult/embryonic stem cells for tissue engineering applications. Of particular interest is leveraging theme expertise in biomaterials fabrication, drug delivery systems, microfluidics-based *in vitro* experimental platforms, and *in vivo* evolutionary biology and regeneration medicine studies. The ideal candidate will have experience in one or more areas of (stem) cell biology, induced pluripotent cell technology, biomaterials, microfluidics, and/or tissue engineering. (Paul Kenis, Theme Leader)

GENOMIC ECOLOGY OF GLOBAL CHANGE

The Fellow will be involved in a cross-disciplinary project investigating how changes in networks of genes affect ecosystem metabolism when challenged by elements of global change, including elevated atmospheric carbon dioxide and ozone, increased drought, and altered interactions with insect herbivores and plant pathogens. The ideal candidate will have a strong background in plant biology and a record of expertise in molecular biology, genomic ecology, physiology or modeling of gene networks or ecosystem function. The ability to work creatively and productively in a highly interdisciplinary and collaborative environment is essential. (Don Ort, Theme Leader)

ENERGY BIOSCIENCES INSTITUTE

The Energy Biosciences Institute (EBI) is an externally funded Theme within the IGB. It is the largest academia collaboration to date, currently receiving \$500 million over the next ten years and focusing on the development of second-generation biofuels intended to significantly slow the rate of global climate change. Its research ranges from systems biology of fermentative organisms to quantification of ecosystem services provided by new sustainable biofuel crops. The full range of research can be seen at www.energybiosciencesinstitute.org. We seek an outstanding candidate across these areas interested in applying genomic biology to understanding and developing opportunities for improving sustainable biofuel production. Research can be at any point in the supply chain from improving feedstocks and their environmental sustainability to producing fuel. The appointee will work in an inter-disciplinary laboratory of over 100 exceptional colleagues focused on this challenge. The appointment would also involve collaboration with our partners: UC Berkeley and BP. (Steve Long, Theme Leader)

MINING MICROBIAL GENOMES FOR NOVEL ANTIBIOTICS

The Fellow will be involved in a multi-disciplinary project aimed at the discovery, design, and development of phosphonic acid antibiotics. The ideal candidate will have a proven record of expertise in the general area of microbially produced natural products, with specific expertise in one of several disciplines. We are interested in candidates with previous experience in bacterial metabolism, bacterial genetics, molecular biology, biochemistry, enzyme evolution, metabolic engineering, organic synthesis, mass spectroscopy, bioinformatics and metagenomics. (Bill Metcalf, Theme Leader)

PRECISION PROTEOMICS

We seek an individual to develop next-generation technologies at the intersection of mass spectrometry and chemical biology. The successful Fellow will have advanced training in mass spectrometry of proteins (with emphasis on "Top Down" techniques and tandem mass spectrometry of high-mass ions), associated proteomics software, capillary separations, and cell culture. The Fellow will work in an interdisciplinary fashion with expert labs in single molecule fluorescence, natural products discovery, and high-throughput screening for anti-cancer compounds. (Neil Kelleher, Theme Leader)

GENOMICS OF NEURAL AND BEHAVIORAL PLASTICITY

We seek a biologist with strong bioinformatics skills and training in one or more of the following areas: evolutionary biology, neuroscience, animal behavior, molecular biology, or genomics. Applicants with expertise in both biology and bioinformatics will be strongly preferred. The successful candidate will join a multi-disciplinary team that is using genomics to identify both conserved and novel mechanisms of neural and behavioral plasticity in diverse animal systems. Fellows are expected to conduct research that contributes to the development of the theme's goals by integrating components from theme members' individual research programs. (Gene Robinson, Theme Leader)

HOST-MICROBE SYSTEMS

The Fellow will be responsible for developing DNA isolation, microbial isolation, 16S rRNA gene library construction and other metagenomic analysis techniques for surveying microbial content of the human and nonhuman primate vaginal and intestinal microbiomes. Additional responsibilities will include the development of microarray and other molecular biology techniques to examine microbial, metabolic, and immunologic contents, and performing analyses using bioinformatics and other computational and analytical methods. The ideal candidate will have a strong background in microbiology, biochemistry, or a related field with experience and expertise in molecular microbial ecology and bioinformatics and/or biostatistics. (Brenda Wilson, Theme Leader)

BUSINESS, ECONOMICS, AND LAW OF GENOMIC BIOLOGY

We seek an individual with training in economics, business, law, or strategy and with an interest in technology entrepreneurship, technology industries, and biotechnology. The Fellow will join a multi-disciplinary group that includes business, law, and technology experts; agricultural economics faculty; and personnel from the campus Office of Technology Management. Our theme is exploring issues in university-industry technology transfer, industry evolution, intellectual property protection, the competitive and cooperative dynamics for both entrepreneurial start-ups and existing corporations, the impact that globalization of biotechnology has on the evolution of industry, and the position of U.S. firms in the global marketplace. (Jay Kesan, Theme Leader)

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Science Careers conducts surveys on the postdoctoral experience every year, alternating between polling postdoc supervisors and the postdocs themselves. This year's survey, which was completed by over 700 postdoc supervisors, coincided with an economic downturn in many countries. It is perhaps not surprising that, when asked about what career trends they were noticing, many supervisors expressed concern about the job market.

"When I was doing a postdoc, I don't remember anyone ever being out of work or not being able to find a position. Whereas now I do hear about postdocs who are looking for work," says **Yvonne Paterson**, dean for postdoctoral research training and director of the biomedical postdoctoral program at the University of Pennsylvania in Philadelphia. Paterson, who completed a three-year postdoc before joining the faculty at Penn in 1982, says she has noticed an increase in the age of first faculty appointments and promotions. "Most faculty we hire have done more than one postdoc," she says.

The data support Paterson's observations. According to the National Research Council's report "Bridges to Independence," the age of first independent faculty appointments for Ph.D.s has been rising steadily from 34 in 1979 to 38 in 2003 (<http://bit.ly/eYIVQ>).

In addition, the share of recent science and engineering doctorate holders hired into full time faculty positions fell from 74 percent to 44 percent from 1972 to 2003, whereas the share of those reporting to be in a postdoc position rose from 13 to 34 percent. (National Science Board, 2006, Science and Engineering Indicators 2006, www.nsf.gov/statistics/seind06/).

And the phenomenon does not seem to be US-specific. "Some people have been postdocs for a very long time, especially if they are in a lab that is well funded," says **Edith Sim**, director of graduate and research staff training for the division of medical sciences at the University of Oxford, UK. "Many postdocs get a faculty position after only one postdoc, but I know many others who have done several postdocs. Some of them have been postdocs for as long as 11 years."

Are Postdocs Getting Longer?

Although it may be a longer haul to an academic position, the length of individual postdoc appointments has actually gotten shorter. When *Science Careers* survey participants were asked the average length of the postdoc experience in their own labs, 67 percent of those polled in 2005 said one to three years and 29 percent said four plus years. In 2007, however, 79 percent said the average postdoc appointment was one to three years and 16 percent four plus years; in 2009, 76 percent said one to three years and 19 percent four plus years. Thus, it seems that the average length of a postdoc stint decreased sharply from 2005 to 2007 and has been fairly constant since then.

One of the factors that may account for shorter postdoc appointments is that many universities and funding bodies in the US and Europe have put in place limits on the length of time a postdoc appointment can last. These limits typically range between three and seven years, sometimes including previous postdoc experiences within that time frame.

Ola Hermanson at the Karolinska Institute in Sweden supports limits on postdoc appointments, but points out that in some cases they can be too restrictive. In Sweden, researchers are normally eligible for assistant professor positions only within five years of obtaining a Ph.D. "Nowadays it takes longer to publish in a good journal. Five years is really not enough time to establish yourself," he says. "Especially because after graduate school you usually take some time before starting a postdoc. So by the time you start, the time you have left to do your research could actually be only three years." **continued »**

"I think there is an increased awareness of the contributions postdocs make."



From Top: Nick Birch, Jonathan Dinman, Lynn Zechiedrich

UPCOMING FEATURES

Faculty Feature:
Funding Your Future — September 11
Focus on Europe — September 25
Top Employers Survey — October 2



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- **Senior Statistical Geneticist (Integrative Computational Sciences)**
- **Senior Medicinal Chemist**
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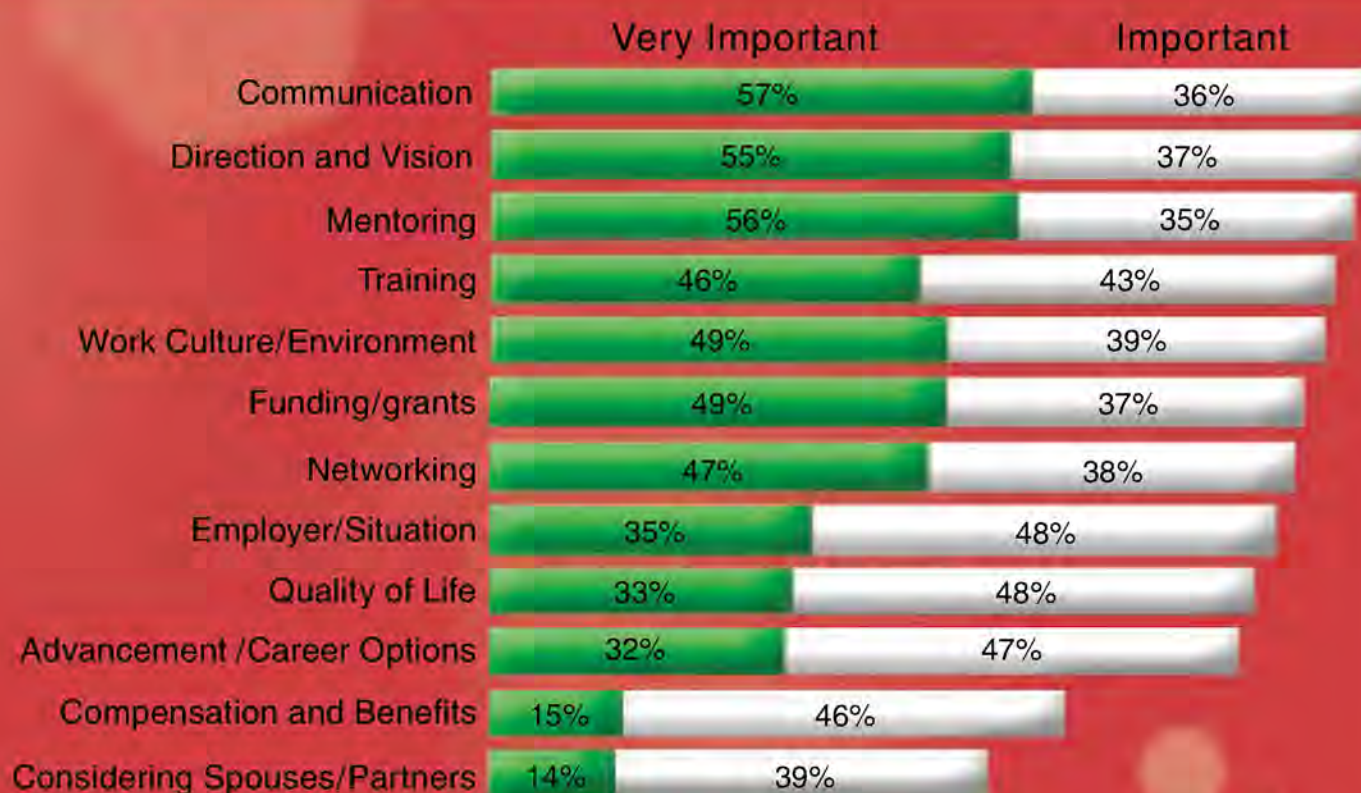
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Answers That Matter.

Postdoc Survey Results

Importance of Attribute Groups in Contributing to a Successful Postdoc Experience



Survey Methodology

This year's survey aimed to determine what factors contribute to a successful postdoctoral experience from the supervisors' point of view. Starting February 3, 2009, 717 postdoc supervisors in North America, Europe, and Asia/Pacific Rim responded to an online survey. In addition to being asked to rate the importance of various attributes to the postdoc experience, supervisors were asked to identify their best postdoctoral fellows and note their strengths. They were also asked to comment about the ways in which the postdoctoral experience had changed in recent years in both positive and negative ways. The majority (87 percent) of survey participants were located in North America (59 percent) and Europe (28 percent). The remainder were located in Asia/Pacific Rim (10 percent) or other areas of the world (3 percent). Most (67 percent) worked in academic institutions, followed by government organizations (9 percent), nonprofit research organizations (7 percent), and hospitals (6 percent).

Another potential disadvantage to short postdocs is that researchers become more focused on getting results fast. "Young scientists need to see the bigger picture," says **Nick Birch**, an entomologist at the Scottish Crop Research Institute in Dundee. "Some research topics may not be in vogue now but will be in 10 years." But, Birch points out, it may be difficult to see a big picture when postdocs are so short. At his own government research institution, postdocs are typically funded for only one to three years, although appointments can be renewed if additional funding is available.

'Alternative' Careers

Another trend that some 2009 survey participants identified is that there are more opportunities for postdocs to pursue outside of academia. "I did my postdoc in the early '90s at the National Institutes of Health. At that time the only jobs were academia or drug companies," says **Jonathan Dinman** at the University of Maryland, College Park.

Postdocs today are able to pursue careers in the biotech industry, government, research advocacy, science writing, intellectual property, and so on. Regardless of the career choice, Dinman points out that it is part of the supervisor's responsibility to offer to support. "I don't have to clone myself," he says. "My job as a mentor is to recognize the strength of each person in my lab and guide them toward their strength."

Although most postdocs begin their training aiming for an academic career, according to informal surveys by the US National Postdoctoral Association (NPA), many end up choosing other options. "Postdocs should consider all career options," says **Cathee Johnson Phillips**, NPA's executive director, adding that NPA is no longer referring to careers outside of academic research as 'alternative' careers. "If the data we have give us an accurate picture, an academic career may actually be the alternative career," says Phillips.

In some countries, though, opportunities for postdocs in academic research might be on the rise. "The intensity of funding is increasing annually and can be available from many sources—the National

Foundation, the local government, and local institutions," says **Jia Wei Zheng** at Shanghai Jiao Tong University in China. "Many Chinese postdocs still go abroad to do research, but nowadays more and more of them come back to China to continue their career."

More Recognition for Postdocs

Another positive trend that 2009 survey participants identified is that postdocs today are more likely to have higher salaries than 10 years ago and to have benefits. According to data from the US National Science Foundation, of the postdocs who received their Ph.D.s between 2001 and 2006, 91 percent received health benefits and 50 percent received retirement benefits from their current or most recent postdoc employer. (National Science Foundation, division of science resources statistics, Postdocs Participation in Science Engineering and Health Doctorate Recipients, 2008, www.nsf.gov/statistics/infbrief/nsf08307)

"I think there is an increased awareness of the contributions postdocs make. And people are more aware of the need to provide benefits. In general, the postdoc community is more in the forefront," says Phillips. "But that does not mean that we don't still have a long way to go."

One of the main factors that has served to increase such awareness is the establishment of postdoc offices and associations at many universities. At the University of Alberta in Edmonton, Canada, the postdoc office has made "the situation between postdoc and supervisor really clear," says biochemistry professor **Joel Weiner**. "Expectations used to be loosely defined. Now we have a formal letter that lays out the project the postdoc will work on, the rules about the maximum length of the appointment, and enforced salary levels."

Under new regulations, postdoc positions at the University of Alberta cannot last longer than five years. "I think it has been a really good change," says Weiner. "But some people don't like it. You can no longer have a postdoc in your lab for six or seven years at \$20,000 a year." **continued »**



The California Nanosystems Institute (CNSI) at the University of California-Santa Barbara is pleased to announce the competition for the ELINGS PRIZE FELLOWSHIPS in EXPERIMENTAL SCIENCE

These prize postdoctoral fellowships provide a salary of \$60,000/yr for two years, renewable for a third, along with benefits and research funds. Successful applicants will work with experimental CNSI faculty; applicants should indicate the experimental group(s) with which they would prefer to work. See www.cnsi.ucsb.edu/fellowships.

Applicants holding a PhD in science or engineering should submit a cover letter, curriculum vitae, a one-page research proposal, and arrange for 3 supporting letters, all to be sent to elingsfellowships@cnsi.ucsb.edu by **December 1, 2009**.

The University of California is an Equal Opportunity/Affirmative Action Employer.

The **Computational Bioscience Program** at the **University of Colorado, Denver School of Medicine** is recruiting to fill **two NLM (NIH) funded postdoctoral fellowships**. The Computational Bioscience Program is home to eight core faculty working in the areas of genomics, text mining, phylogenetics, network analysis, statistical methods, microarray analysis, biomedical ontology, and other areas. The School of Medicine is home to a broad array of outstanding research and instrumentation, including a 900 MHz NMR, extensive DNA sequencing and microarray facilities, and more. We are housed on the first all-new medical campus of the 21st century, close to both the urban amenities of Denver and the beautiful Rocky Mountains. For more information, please consult <http://compbio.uchsc.edu>.

QUALIFICATIONS: Candidates must have a Ph.D. degree in Computational Biology or a related discipline, and be **U.S. Citizens or Permanent Residents**.

SALARY/BENEFITS: Successful candidates will be offered the NRSA specified stipend (based on years of experience), medical insurance, \$2000 per year in travel support and \$6500 per year in additional research-related expenses.

TO APPLY: Send to **Kathy.R.Thomas@ucdenver.edu** a cover letter, curriculum vitae, and a statement of research interests; also arrange for three letters of recommendation to be sent to the same email address.

UC Denver is an Equal Opportunity Employer. Women and minorities are especially encouraged to apply.



POSTDOCTORAL POSITIONS

The Research Foundation of Stony Brook University/SUNY anticipates the following postdoctoral positions being available between Summer 2009 and December 2009.

• BIOCHEMISTRY AND CELL BIOLOGY

Role of O-linked glycosylation in signaling and development. Robert Haltiwanger, WC-R-5927-09-08-S

Role of RNA-protein interactions in bacterial pathogenesis. A.Wali Karzi, WC-R-5929-09-09-08-S

Biochemistry of glycoproteins. William J. Lennarz, WC-R-5928-09-08-S

Structure of G protein-coupled receptors by NMR and molecular dynamics.

Steven O. Smith, WC-R-5926-09-08-S

• CENTER OF EXCELLENCE IN WIRELESS AND INFORMATION TECHNOLOGY (CEWIT) AND ECONOMIC DEVELOPMENT

Conduct research and work on R&D/Commercialization projects with sponsors, partners, and new start-ups, end-to-end solutions. Satya Sharma, WC-R-4297-09-08-S

• CHEMISTRY

Ultra-nano filtration/reverse osmosis, water purification, multifunctional copolymers, polymer inorganic hybrids. Ben Chu, WC-R-5925-09-08-S

Polymer synthesis, nanocomposites, ultrafiltration/nanofiltration/reverse osmosis.

Benjamin S. Hsiao, WC-R-5924-09-08-S

• MATERIALS SCIENCE AND ENGINEERING

Science and technology of thermal spray processing and related materials.

Sanjay Sampath, WC-R-5914-09-08-S

• MEDICINE AND CANCER PREVENTION

Dissect role of C1q in dendritic cell differentiation and function.

Berhane Ghebrehwet, WC-R-5922-09-08-S

Evaluate nove nanosensors for detection of disease markers. Basil Rigas, WC-R-5933-09-07-S

Evaluate pharmacokinetic and pharmacodynamic features of novel anti-cancer/anti-inflammatory agents.

Basil Rigas, WC-R-5934-09-07-S

Evaluate molecular targets and cell-signaling cascades of novel anti-cancer/anti-inflammatory agents.

Basil Rigas, WC-R-5935-09-07-S

• MOLECULAR GENETICS AND MICROBIOLOGY

Molecular virology: hantavirus and dengue pathway regulation and pathogenesis.

Erich R. Mackow, WC-R-5891-09-07-S

Molecular virology: influenza virus pathogenesis. Erich R. Mackow, WC-R-5892-09-07-S

Genetic engineering and tissue culture related to poliovirus. Eckard Wimmer, WC-R-5936-09-08-S

• NEUROBIOLOGY AND BEHAVIOR

Behavioral neurophysiology of taste and olfaction in rodents. Alfredo Fontanini, WC-R-5908-09-08-S

Experiments aimed at examining the regulatory mechanisms of adult neurogenesis.

Shaoyu Ge, WC-R-5904-09-08-S

Molecular/cellular neuroscience. Simon Halegoua, WC-R-5905-09-08-S

Experience-dependent plasticity of neocortical circuits. Arianna Maffei, WC-R-5909-09-08-S

Expression of optogenetic photosensors in the retina. Gary Matthews, WC-R-5906-09-08-S

Structure and function of synapses in the retina. Gary Matthews, WC-R-5907-09-08-S

• PHARMACOLOGY

Breast cancer metastasis: organ-specific metastasis, hypoxia shotgun proteomics.

Emily Chen, HS-R-5923-09-07-S

Mammalian DNA repair pathways. Michael Frohman, WC-R-5893-09-08-S

Mammalian signal transduction: lipid signaling, diabetes, mitochondrial dynamics,

cancer, and secretion. Michael Frohman, WC-R-5894-09-08-S

• PHYSICS AND ASTRONOMY

Research in theoretical nuclear physics. Edward Shuryak, WC-R-5895-09-08-S

To apply online and for information, visit www.stonybrook.edu/jobs or mail résumés to: Margaret Rothwell, Office of the President, Stony Brook University, Stony Brook, NY 11794-0701.

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Postdoc Survey Results

PI Rankings 2009	Postdoc. Rankings 2008	Attribute
1	7	Communication
2	1	Direction and Vision
3	4	Mentoring
4	9	Training
5	N/A	Work Culture/Environment
6	2	Networking
7	5	Employer/Situation
8	N/A	Quality of Life
9	6	Advancement/Career Options
10	8	Compensation and Benefits
11	10	Considering Spouses/Partners

Comparison of attribute rankings from principal investigators (PIs) and postdocs. The table compares certain attributes of a successful postdoc, as rated by PIs in this year's survey, and postdocs in the 2008 survey. Of note are the clearly divergent views on Communication, Networking, and Training.

Lawrence Livermore National Laboratory in California, a government laboratory funded primarily by the US Department of Energy, hires about 50 percent of its postdocs as permanent staff members. **Ian D. Hutcheon**, deputy director of the Glenn T. Seaborg Institute at Lawrence Livermore, has noticed that in recent years there are more opportunities for women to be postdocs and then move on to obtain permanent positions. "We have a pretty generous maternity leave policy; there is much greater awareness of the needs for all postdocs to spend time with their families," says Hutcheon.

This awareness was lacking when **Lynn Zechiedrich** did her postdoc at the University of California, Berkeley in the early 1990s. "I did not know whether I even had any benefits until I became pregnant," she says. "I just did not think about it. I was focused on my work." When her son was just seven weeks old Zechiedrich was asked to return to work. "It never occurred to me I could ask for more time," she says. "Today postdocs are not so clueless. They will speak out."

The change, Zechiedrich and others believe, has to do with postdocs being savvier and more aware, as well as a general change in culture. "There are more women in science, more families, and more acceptance of family," says Zechiedrich, who is at the Baylor College of Medicine in Houston, Texas. "There is also more appreciation of the work being done by trainees and willingness to treat them well."

Where Have the Outstanding Postdocs Gone?

One change from this year's survey compared to the one conducted in 2007 is that only 39 percent of supervisors said they are currently supervising a postdoc that they consider to be outstanding—down from 45 percent in 2007.

In some of the "newer" research fields, outstanding postdocs are still hard to come by. "In our particular field, bioinformatics, it is rare to find postdocs with really strong skills both in the life sciences and in a technical area. For example, a mathematician who is able to formulate and pursue a biologically relevant research question or someone with lab experience who is able to develop new models or algorithms," says **David Kreil** of Boku University in Vienna. He points out that most current Ph.D. programs do not provide this kind of "dual-training."

Most postdoc supervisors who were interviewed for this article, however, did not think that the quality of postdocs had changed. "I have not seen a decrease in the availability of qualified people but we all like to hire the best and the brightest. It's a very competitive market. We all try to hire people who are really good but there are only so many of them," says Hutcheon.

So what should a supervisor look for? The most common attribute 2009 survey participants looked for when recruiting a new postdoc was a strong research experience; it was cited by 82 percent of those polled. Other common factors included interest in working in new fields (53 percent), having a graduate adviser with a good reputation (48 percent), and coming from a good research institution (34 percent).

One thing that has not changed in this year's survey compared to the 2007 one is what supervisors believe makes a successful postdoc experience. When survey participants were asked to rank the importance of several factors, the majority agreed that conducting high quality research (79 percent), learning to work independently (66 percent), and publishing work (65 percent) were most important to a successful postdoc experience. These responses were virtually unchanged from two years prior.

Similarly when participants were asked to rate the importance of 12 groups of attributes in contributing to a successful postdoc experience, the three that came up on top—communication (91 percent), direction and vision (92 percent), and mentoring (91 percent)—were also the same as those ranked highest in 2007 but differed in certain aspects from 2008 postdoc rankings (see table, left).

Scientific careers have undergone many changes in recent years. Some changes have to do with the funding situation, others with the type of research that is being conducted, others yet, with the rules and regulations put in place at institutions. But one thing that will probably never change is why people do science. "If you don't love doing science, you should never pick this career," says Hermanson. "We live for the moment of discovery. Three hundred sixty days could be pretty boring. But it's definitely worth it for those 5 days of excitement."

Featured Participants

Baylor College of Medicine
www.bcm.edu

Boku University
www.boku.ac.at/home.html?L=1

Glenn T. Seaborg Institute
seaborg.lanl.gov

Karolinska Institute
ki.se/?l=en

National Postdoctoral Association (NPA)
www.nationalpostdoc.org

National Research Council
www.nationalacademies.org/nrc

Scottish Crop Research Institute
www.scri.ac.uk

Shanghai Jiao Tong University
www.sjtu.edu.cn/english/index

University of Alberta
www.ualberta.ca

University of Maryland, College Park
www.umd.edu

University of Oxford
www.ox.ac.uk

University of Pennsylvania
www.upenn.edu

Laura Bonetta is a scientist turned freelance writer based in the Washington, D.C., area.

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Postdoctoral Research Positions—Computational Phylogenetics/Phyloinformatics

Multiple postdoctoral positions are available for research and cyberinfrastructure development associated with reconstructing the green plant tree of life. Positions are sponsored by the iPlant Collaborative (<http://iplantcollaborative.org>), which is supporting cyberinfrastructure across the plant sciences. Individuals would work with one of a number of identified investigators focusing on specific issues in phylogenetic cyberinfrastructure development. These include high performance computing and scalable tree construction (**Alexandros Stamatakis**—Technical University, Munich; email: stamatak@cs.tum.edu), data set assembly (**Doug Soltis**—U Florida; email: dsoltis@botany.ufl.edu, **Pam Soltis**—U Florida; email: psoltis@flmnh.ufl.edu; **Michael Donoghue**—Yale; email: michael.donoghue@yale.edu), gene tree reconciliation (**Todd Vision**—UNC; email: tjv@bio.unc.edu), character evolution (**Brian O'Meara**—U Tennessee; email: bomeara@utk.edu), and tree visualization (**Michael Sanderson**, UC-Davis; email: sanderma@email.arizona.edu). Fellows can anticipate working in a highly collaborative, multi-institutional context, with some travel between working groups encouraged. In addition to undertaking basic research in phylogenetic methods, postdocs will be expected to provide advice on and work in collaboration with cyberinfrastructure developers in the iPlant team. Qualifications include familiarity with the data, methods, and software of phylogenetic analysis, and programming experience at least at the level of a scripting language such as PERL. Individual PIs may have additional requirements. Inquiries should be directed to any of the Grand Challenge Team members listed above. Positions are available immediately. A full description of the iPToL (iPlant Tree of Life) project can be found online at <http://iptol.iplantcollaborative.org>.

The institutions involved in this program may be Affirmative Action and/or Equal Opportunity Employers.

Program Announcement—Connecting Genotypes to Phenotypes in Complex Environments

Multiple postdoctoral positions will be available for research and cyberinfrastructure development associated with elucidating the relationship between plant genotypes and the resultant phenotypes (G2P) in changing environments. The program is sponsored by the iPlant Collaborative (<http://iplantcollaborative.org>), which develops and enables cyberinfrastructure support for Grand Challenge research across the plant sciences. Individuals will work in a team setting with one of a number of specific investigators having key roles in issues related to G2P cyberinfrastructure development. These include pipelining and analysis of Next Generation sequence data (**Tom Brutnell**—Boyce Thompson Institute, Cornell University; email: tpb8@cornell.edu), data integration (**Matthew Vaughn** email: vaughn@cshl.edu, and **Doreen Ware** email: ware@cshl.edu, — both at Cold Spring Harbor Laboratory), statistical inference for genome-wide association mapping (**Dan Kliebenstein**—University of California, Davis; email: kliebenstein@ucdavis.edu), visualization/analysis (**Ruth Grene**—Virginia Tech; email: grene@vt.edu), modeling tools (**Stephen Welch**—Kansas State University; email: welchsm@ksu.edu). The work setting will be a highly collaborative, multi-institutional context, with some travel between working groups encouraged. In addition to undertaking basic G2P research, individuals will be expected to provide advice on and work in collaboration with iPlant cyberinfrastructure developers. Qualifications include familiarity with one (or preferably more) of the following areas: functional-, quantitative-, or computational genetics/genomics; bioinformatics; programming experience (either scripting languages like PERL or compiled languages like C/C++); modeling (systems biological, ecophysiological, or statistical); database use; current topics in plant biology (especially stress, photosynthesis, or phenology); or high performance computing. Individual investigators may have additional requirements. Inquiries should be directed to any of the individuals listed above. A full description of the iPlant G2P program, including specific position descriptions as they become available, can be found online at <http://ipg2p.iplantcollaborative.org>.

The institutions involved in this program may be Affirmative Action and/or Equal Opportunity Employers.

POSITIONS OPEN



CALIFORNIA INSTITUTE OF TECHNOLOGY Positions in Neuroscience

The California Institute of Technology invites applications for two tenure-track professorial positions in the Division of Biology. A position in **Systems Neuroscience** is open to investigators studying the neural mechanisms and circuit properties that underlie behavior. A position in **Cellular and Molecular Neuroscience** is aimed at applicants whose research program concerns molecular and/or cellular aspects of physiology, behavior, or neural development. Successful applicants are expected to develop innovative research programs and should also have a commitment to undergraduate teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Appointment is contingent upon completion of Ph.D.

Please submit on-line application at <http://biology.caltech.edu/Positions> and include a brief cover letter, curriculum vitae, relevant publications, and a description of proposed research. Applicants should indicate for which of the two positions they wish to be considered. Instructions will be given for submission of letters of reference when you apply on-line. Applications will be accepted until the position is filled.

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POSTDOCTORAL OPPORTUNITIES



THE UNIVERSITY OF BRITISH COLUMBIA

UBC POSTDOCTORAL POSITIONS IN FUNCTIONAL GENOMICS OF TERPENE SYNTHASES AND CYTOCHROME P450 ENZYMES

Michael Smith Laboratories, University of British Columbia, Canada

Genome Canada, with Genome BC, will fund new functional genomics research on terpenoid synthases and P450 enzymes of plant defense and natural products biosynthesis starting in October 2009. The positions are part of a long-term research program on terpenoid biosynthesis, terpenoid metabolic engineering, and conifer defense against insects (www.michaelsmith.ubc.ca/faculty/bohlmann/; e.g.: *PNAS* 106:7245; *PNAS* 105:1085; *PNAS* 102:8060; *The Plant Journal* 54:656). Applicants must have a recent PhD in plant biology, molecular biology, biochemistry, chemistry, or a related area. The ideal candidate will have at least 2 years of experience in molecular biochemistry of terpenoid synthases or P450 enzymes. Experience with analysis of 454 sequence data, transcriptome, proteome, or metabolome analyses will be strong assets.

Interested candidates should apply with a CV, statement of research interest, and names of three references to: **Professor Jörg Bohlmann** (bohlmann@msl.ubc.ca).



UNBC UNIVERSITY OF NORTHERN BRITISH COLUMBIA

UNBC POSTDOCTORAL POSITIONS IN ENTOMOLOGY AND POPULATION GENOMICS

Ecosystem Science and Management, University of Northern British Columbia, Canada

Genome Canada, with Genome BC, will fund new research on mountain pine beetle biochemistry and population genomics starting in October 2009. The positions are part of a large-scale research program on the mountain pine beetle (MPB) epidemic (www.thetriproject.ca):

Entomology (Dr. Dezene Huber) – 2 positions: (1) Functional characterization of MPB gene products related to detoxification of host secondary metabolites: example genes include cytochromes P450, carboxylesterases, and glutathione S-transferases. Successful candidates should be familiar with insect, yeast and bacterial expression systems and able to perform functional characterization enzyme assays. (2) Molecular genetic mechanisms of cold tolerance in MPB: successful candidates should have field and lab research skills, including preparation of samples for RT-qPCR, microarray and proteomic analyses. The ability to design, plan, and carry out replicated, ecologically based experiments is a necessary qualification.

Population Genomics (Dr. Brent Murray) – 1 position: (3) Investigating the integrated landscape genomics of the MPB system, this position focuses on the study of neutral (microsatellite) variation among collections of MPB populations. Previous experience with field collection and population genetic/genomic analysis is an asset.

Noting position for application, apply with CV and names of three references to: **Project Director Kyeema Burns** (kyeema@interchange.ubc.ca).

All qualified persons should apply; however, Canadians/permanent residents of Canada are given priority. Only qualified applicants will receive a reply.

POSTDOCTORAL OPPORTUNITIES



**Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Postdoctoral Research/Regulatory Opportunities**

Interested in cutting-edge science? Then consider our Service Fellowship Program!

The Center for Biologics Evaluation and Research's mission is to protect and enhance the public health through the regulation of biological products including blood, vaccines, allergenics, tissues, and cellular and gene therapies. Biologics, in contrast to drugs that are chemically synthesized, are derived from living sources (such as human, animals, and microorganisms) and are not easily identified or characterized, and many are manufactured using biotechnology. These products often represent cutting-edge biomedical research and, in time, may offer the most effective means to treat a variety of medical illnesses and conditions that presently have few or no other treatment options.

To accomplish its mission, CBER seeks candidates whose expertise covers a broad range of scientific disciplines and whose responsibilities include the mission-oriented research and regulation of biological products.

Qualifications: Staff Fellow and Senior Staff Fellow applicants must be U.S. citizens. Non U.S. citizens holding a valid U.S. employment visa are eligible for Visiting Associate and Visiting Scientist positions. These positions may also be filled by appointment in the US Public Health Service, Commissioned Corps.

- **Staff Fellow** - must possess a Ph.D. or equivalent degree (e.g., MD, VMD, or Sc.D) plus less than 2 years of post-doctoral health-related research/regulatory review experience.
- **Senior Staff Fellow** - Must possess a Ph.D. or equivalent degree (e.g., MD, VMD, or Sc.D) plus 2 or more years of post-doctoral health-related research/regulatory review experience

Candidate must either have been awarded their doctoral degrees in a bio-medical, behavioral, or related science or have been certified by a university as meeting all the requirements leading to such a doctorate.

Salary Range: Salary equivalent to GS-11/13 (\$60,989 - \$113,007). Salary is commensurate with education and experience.

How to Apply: For current vacancies and to apply, visit: <http://www.fda.gov/AboutFDA/CentersOffices/CBER/ucm103202.htm>. For future vacancies, submit CV/resume and cover letter to our resume bank by **October 30, 2009** to: CBER.Employment@fda.hhs.gov Attn: Postdoc

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Vanderbilt University Medical Center

Pharmacology Faculty Positions in Drug Discovery

The Department of Pharmacology and the Vanderbilt Program in Drug Discovery in the **Vanderbilt University School of Medicine** is recruiting faculty in the area of CNS drug discovery, with specific interest in individuals in the areas of **Drug Metabolism, Pharmacokinetics, and/or in vivo neuropharmacology**. We invite applications for faculty positions from individuals interested in issues related to drug disposition, especially as they relate to activity of drugs acting in the CNS. Also, we welcome applications from candidates interested in behavioral pharmacology or other areas of in vivo neuropharmacology who are interested in working in a team-oriented academic drug discovery setting.

Candidates should send curriculum vitae, description of research interests and accomplishments to:

**P. Jeffrey Conn, Professor
Department of Pharmacology
Vanderbilt University School of Medicine
1215D Light Hall
Nashville, TN 37232-6600
p: (615) 936-2189 f: (615) 343-3088
E-mail: jeff.conn@vanderbilt.edu**

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POSTDOCTORAL OPPORTUNITIES



Omidyar Fellowships at the Santa Fe Institute

The Omidyar Fellows Program attracts the brightest and most creative thinkers to spend two to three years as postdoctoral-level fellows at the Santa Fe Institute (SFI). Consistent with SFI's multidisciplinary approach, the fellowship program will draw scholars from across the social, physical and natural sciences. The common denominator among all SFI scientists is intense curiosity, creativity and a desire to delve deeply into the major questions facing science and society.

Applications are welcome from candidates in any country. Women and minorities are especially encouraged to apply.

Deadline: **November 2, 2009**

For further information and to apply:

www.santafe.edu/education/fellowships.php

Inquiries: email ofellowshipinfo@santafe.edu

The Santa Fe Institute is an Equal Opportunity Employer



SANTA FE INSTITUTE

DEPARTMENT OF BIOENGINEERING



FACULTY POSITIONS

The Department of Bioengineering in the School of Engineering and Applied Science at the University of Pennsylvania invites applications for both tenure track and tenured faculty positions at all professorial levels. The University of Pennsylvania provides unique opportunities for multidisciplinary research. Bioengineering is prominent in the strategic plans of the School of Engineering and Applied Science and the University emphasizing integrative research in all biomedical areas ranging from fundamental to translational research. Bioengineering at Penn has collaborations and research connections spanning the medical school and hospitals, as well as many outstanding departments in the University's other schools, centers and institutions, (including, for example, the Institute for Medicine and Engineering and Institute for Neurological Sciences). All of Penn's schools are situated on one campus making it an ideal environment for multidisciplinary research. Bioengineering seeks to enhance its integrative research and educational programs. We are especially interested in energetic visionary faculty who are committed to developing a research enterprise in this environment.

Candidates should hold a doctoral degree in Bioengineering/Biomedical Engineering or a related field. At the assistant professor level, individuals should demonstrate superb academic credentials and promise for a career in bioengineering research. At higher professorial levels, an evident track record of research excellence including successful competitive research funding and academic standing is expected. Growth areas for our program include **cellular and molecular engineering, systems/synthetic biology, optical imaging, neuroengineering, and orthopedic bioengineering**. However, we encourage applications from candidates in any emerging area of bioengineering. A commitment to teaching should also be apparent including development of undergraduate and graduate courses, supervision of doctoral students and academic advising of students at all levels.

Please submit all applications to:

<http://facultysearches.provost.upenn.edu/applicants/Central?quickFind=50706>

Applications will be considered until November 1, 2009 or until filled.

The University of Pennsylvania is an EOE. Minorities/ Females/Individuals with Disabilities/Veterans are encouraged to apply.



**Tenure Track/Tenured Position
Basic Biomedical Research**

The Division of Intramural Research (DIR) of the National Heart, Lung and Blood Institute (NHLBI) is seeking an outstanding scientist to initiate and direct an independent research program in basic science on the NIH campus in Bethesda, MD. The area of expertise of the candidate is less important than his/her demonstrated ability to conduct outstanding independent research in areas within the broad biomedical research interests of the DIR. The areas of expertise may include but are not limited to: cell biology, developmental biology, molecular biology, immunology, stem cell biology, genetics, genomics, physiology, biochemistry, bioenergetics and metabolic regulation, biophysics, biomolecular structure and dynamics, systems and synthetic biology, and biomedical engineering. Technical approaches may include: high-resolution microscopy, x-ray spectroscopy and imaging, clinical robotics, nanotechnology, chemical biology, computational, and theoretical methods. The existing faculty is an outstanding group of internationally recognized biomedical researchers covering a wide range of basic and clinical research topics (please see <http://dir.nhlbi.nih.gov/> complemented by the other research institutes within the DIR (please see <http://www.nih.gov/science/#campus>).

The DIR environment provides the opportunity to perform creative and innovative science unconstrained by traditional support systems available at academic or private research institutions. This is enhanced by outstanding research core facilities in optical and electron microscopy, transgenic and knockout mouse production, mouse phenotyping, proteomics, genomics, and flow cytometry/cell sorting as well as world-class seminar series and symposia. DIR faculty can participate in Graduate Partnerships Programs (<http://gpp.nih.gov>) with many academic programs around the world as a means of recruiting graduate students, and extensive career guidance and development resources are available on campus for post-doctoral trainees.

Candidates must have an M.D., Ph.D., or both and have an outstanding record of research accomplishments as evidenced by publications in major peer-reviewed journals. The position can be filled as a tenure-track or tenured position, but preference will be given to senior post-doctoral fellows or faculty who are still in the early stages of their research careers. The successful candidate will be offered a competitive salary commensurate with experience and qualifications, and will be assigned ample research space, supported positions, and an operating budget. Appointees may be US citizens, resident aliens, or non-resident aliens with or eligible to obtain a valid employment authorized visa. Complete applications must be received by November 1, 2009. Please submit a curriculum vitae, brief (not to exceed 3 pages) statement of research interests and three letters of reference in .pdf or MS word format only (no paper applications will be accepted) to:

Robert S. Balaban, Ph.D., Scientific Director, NHLBI, c/o Michelle Renehan, nhlbi_dir_search@mail.nih.gov.



**Tenure-Track Developmental Biologist
Research Triangle Park, North Carolina**

A position is available for a Developmental Biologist to establish an independent basic research program and form a research group in the Laboratory of Reproductive and Developmental Toxicology, Division of Intramural Research. Applications are invited from scientists with demonstrated ability for creative and productive research in cellular and molecular mechanisms of mammalian development. Of particular interest are investigators using rodent models to study cell interactions, epigenetics or other basic biomedical problems relating to the impact of the environment on development. The successful candidate will interact with investigators studying diverse problems in reproductive biology, developmental toxicology, hormone mechanisms, signal transduction, cell cycle regulation, cell growth and differentiation, apoptosis, gene regulation, mutagenesis and DNA repair, and cancer biology.

Minimum qualifications are an M.D., Ph.D., D.V.M. or equivalent doctoral degree in the biomedical sciences, at least three years of postdoctoral experience, and publications in high quality journals. Salary will be commensurate with the experience and qualifications of the candidate. Federal benefits apply. Information about the NIEHS may be found at www.niehs.nih.gov and the Laboratory of Reproductive and Developmental Toxicology at www.niehs.nih.gov/research/atniehs/labs/lrdt/index.cfm. Applications from women and minorities are encouraged. Interested applicants should provide a curriculum vitae and bibliography, a 2-5 page statement of current research interests and future plans, and have three letters of recommendation sent directly to the address below by **October 30, 2009**. NOTE: Priority consideration will be given to applications **received** by the closing date. Late applications received after the closing date will only be reviewed if needed.

Ms. Lisa Rogers (DIR-09-02)
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop A2-06
111 Alexander Drive, Room A208
Research Triangle Park, NC 27709
e-mail: dir-appls@niehs.nih.gov
<http://dir.niehs.nih.gov>



DHHS and NIH are Equal Opportunity Employers.



**DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF DIABETES DIGESTIVE
AND KIDNEY DISEASES
INTRAMURAL RESEARCH PROGRAM**

Postdoctoral Fellowship

POSTDOCTORAL FELLOWSHIP in Molecular Genetics at the Department of Health and Human Services (DHHS), National Institutes of Health (NIH), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), in Phoenix, AZ. We are working to identify and characterize novel genes that cause type 2 diabetes and obesity in humans. Applicants must have a Ph.D. or M.D. degree obtained within the past 5 years, with research experience in molecular biology. Please send curriculum vitae to **Leslie Baier, Ph.D. 445 North 5th Street, Suite 210, National Institutes of Health, Phoenix, AZ 85004. email: lbaier@phx.niddk.nih.gov.**

NEUROSCIENCE FACULTY POSITIONS

As part of a major research growth initiative, The University of Wisconsin-Milwaukee is seeking applicants for multiple tenure-track faculty positions in the neurosciences. These new faculty will build directly on the current strength of our program (neuroscience.uwm.edu). We expect that most of these appointments will be made at the Assistant Professor level.

Candidates in **Behavioral Neuroscience** should employ modern technical approaches to address important questions related to motivation, development, learning and memory, or neurological disorders. Successful applicants in **Cognitive Neuroscience** will participate in the Functional Imaging Research Center (www.firc.mcw.edu) which provides multiple research-dedicated imaging platforms and technical infrastructure for brain mapping in human subjects and laboratory animals. Area of research specialization is open but outstanding individuals with interests in perception, language, memory, or emotion are particularly encouraged to apply.

Applicants must have a Ph.D. in neuroscience or a closely related field and significant postdoctoral research experience. Responsibilities include developing an independent extramurally funded research program and teaching graduate and undergraduate courses in the neurosciences and experimental psychology. Review of applications will begin on **October 15, 2009** and continue until the positions are filled.

To apply for Behavioral Neuroscience please see:
www.jobs.uwm.edu/applicants/Central?quickFind=51037

To apply for Cognitive Neuroscience please see:
www.jobs.uwm.edu/applicants/Central?quickFind=51038

A complete application will consist of a cover letter, vita, a concise statement of research interests, copies of published work, and three letters of reference. All application materials may be submitted electronically, except applicants should arrange for three letters of reference to be mailed to: **Neuroscience Search Committee, Department of Psychology, UWM, PO Box 413, Milwaukee, WI 53201.** This position is contingent upon budgetary approval.

UWM is an Equal Opportunity Institution committed to diversity.



Washington University in St. Louis

SCHOOL OF MEDICINE

RESEARCH FACULTY POSITION IN ANESTHESIOLOGY

The Department of Anesthesiology at Washington University School of Medicine in St. Louis invites applications for a tenure-track position in its Division of Basic Research. Candidates must have a doctoral degree and postdoctoral training experience. Physician scientists are also encouraged to apply. Applications for both junior and senior faculty positions will be considered. Current scientific fields of interest include: Ion channel/receptor electrophysiology, G-protein signaling, Hypoxic and Immune-mediated cell death mechanisms. These fields as well as neurotoxicity, sleep, and pain are targets of the search. The successful candidate will be expected to direct an active extramurally funded research program and participate in graduate or medical education. Generous startup funds and newly renovated lab space are available.

To apply, send a curriculum vitae, a summary of past research activities, future research plans, and the names of three references to: **Dr. C. Michael Crowder, Director, Division of Basic Research, Department of Anesthesiology, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8054, St. Louis, MO 63110** or email to Lisa Hayes – hayesl@wustl.edu

Equal Opportunity Employer M/F/D/V.



CASE WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE

Faculty Positions Department of Physiology and Biophysics

The Department of Physiology and Biophysics is undergoing major expansion. We invite outstanding individuals to apply for faculty positions at the level of Assistant Professor (tenure track), Associate Professor, and Professor. The Department's major areas of focus are integrated systems physiology, cellular physiology/membrane biology, and protein biophysics/structural biology. We especially encourage applicants who are using interdisciplinary approaches to work on basic or translational aspects of human diseases—particularly in the cardiovascular, renal, and neuronal systems. Visit our website at <http://Physiology.case.edu> or <http://Biophysics.case.edu>.

Applicants for a position as Assistant Professor should have a Ph.D. and/or M.D. degree, 3-5 years postdoctoral experience, and a strong record of scholarly activity. Candidates for more senior positions should be nationally recognized scholars and have a strong, externally funded research program.

Applicants should submit a cover letter and a full *Curriculum Vitae*. Applicants for Assistant Professor positions should also include a brief description of their research plans as well as the contact information for three professional references. Please submit application materials with separate file attachments by email to:

Walter F. Boron, M.D., Ph.D.
Chairman
PhysiologyBiophysicsSearch@case.edu

"In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. CWRU is a recipient of a National Science Foundation ADVANCE Institutional Transformation Grant to increase the participation of women in Science and Engineering."

GRANTS

PAKISTAN-U.S. SCIENCE AND TECHNOLOGY COOPERATION PROGRAM ANNOUNCES NEW CALL FOR PROPOSALS

APPLICATION DEADLINE: October 31, 2009

The Pakistan-U.S. Science and Technology Cooperation Program invites joint applications from Pakistani and American scientists, engineers, and health professionals interested in working together to build capacity in Pakistan and strengthen cooperative relationships.

Projects should be aimed at

1. Improving the quality, relevance, or capacity of education and research at Pakistani institutions of higher education in science and technical fields;
2. Improving the capacity of Pakistani public and private science institutions to support industry competitiveness; or
3. Increasing the capacity of science and technology to improve the well-being of ordinary Pakistani people.

Since 2005, with funding provided by both the Pakistani and U.S. sides, the program has awarded grants to 46 projects ranging from research on diseases affecting humans, animals, and plants to studies on water resources, climate change, telemedicine, earthquake engineering, and information technologies.

Application forms and instructions are available at:

www7.nationalacademies.org/DSC/PAKUS.html

Southern China Center For Innovative Pharmaceuticals

Southern China Center for Innovative Pharmaceuticals (SCCIP) is the largest national new drug research organization in Southern China. It is located in Guangzhou City, the capital of Guangdong Province.

Initiated and supported by the Governments of Guangdong Province and Guangzhou City with around 700 million Chinese Yuan for the first three years, SCCIP aims to build a world-class pharmaceutical R&D infrastructure platform to promote new drug development and innovation. To achieve its objectives, SCCIP seeks to cooperate with any individual or entity which is in the process of developing a new drug and which has not registered the new drug in China.

Related news about SCCIP can be found on "Science" magazine at **Vol 325 on 3 Jul 2009**.



To build a full-fledged center for new drug research and development, SCCIP attempts to attract top talents world-wide. We provide a wide range of support from funding, premises, facilities, technology, scale-up, IND application, SFDA new drug registration to HR services – indeed anything you will need to turn your entrepreneurial dream into reality. At SCCIP, you can focus on what you are best at, i.e. discovering and developing new drugs, away from the hassles or trivia which are usually associated with running a start-up. We believe that in making you successful, we become successful.

We are seeking **Distinguished Scientists in the following fields:**

Principal Investigators and Department Heads

- Synthetic Organic Chemistry
- Medicinal Chemistry
- High Through-put Drug Screening
- Pharmacodynamics
- Drug metabolism & Pharmacokinetics
- Drug Safety Evaluation
- Drug Formulation
- Recombinant Virus & DNA Vaccine
- Recombinant Protein & Antibody
- Genetic Engineering Vaccine
- Polysaccharide Conjugated Vaccine
- Other fields in Pharmaceutical R&D

Minimum requirements

- Ph.D. in related field
- Above 5 years working experience in R&D at a Pharmaceutical Company
- Lead a R&D team
- Willing to be a team-player

Preferred consideration

- Hold proprietary technologies
- Hold own novel drug patents or drug candidates
- Work full-time

Contacts:

Tel: +86-20-28069136

Fax: +86-20-28069137

www.sccip.org.cn

We welcome interested applicants to email their detailed resume plus one or more references, stating preferred area of interest, to **Ms. Crystal Huang (aihuahuang@sccip.org.cn)**.



NMR SPECTROSCOPIST

St. Jude Children's Research Hospital (SJCRH), a premier center for biomedical investigation located in Memphis, Tennessee, USA, is seeking an NMR spectroscopist to fill a faculty position in the Department of Structural Biology. Research in the Department centers on understanding the molecular basis of biological processes with emphasis on mechanisms associated with catastrophic diseases of children, such as cancer. Examples of ongoing research include mechanistic studies of protein degradation, cell cycle regulation, tumor suppressor function, signal transduction, transcriptional regulation, DNA repair, structure-based drug design, and lipid metabolism. A wide range of structural techniques are available, including NMR spectroscopy, X-ray crystallography, and high-level computing.

SJCRH is a hospital and basic research institute that focuses on the fundamental causes and treatment of catastrophic childhood diseases including cancer, infectious diseases and genetic disorders. Founded by Danny Thomas in 1962, the hospital includes some 150 basic and clinical investigators organized into a traditional academic environment. The research environment at SJCRH is highly interactive, with opportunities to collaborate with investigators in other departments, including Biochemistry, Chemical Biology and Therapeutics, Developmental Neurobiology, Genetics and Tumor Cell Biology, Hematology, Oncology, Immunology, Infectious Diseases, Molecular Pharmacology, Pathology and Pharmaceutical Sciences. All investigators have access to state-of-the-art core facilities that include proteomics, genomics, bioinformatics, imaging, protein production, molecular synthesis and high throughput small molecule screening. SJCRH is a National Cancer Institute Comprehensive Cancer Center and continues to receive support through the fundraising efforts of the American Lebanese Syrian Associated Charities (ALSAC).

The candidate will be expected to develop an independent and funded research program and to eventually become an established investigator. He/she will have interests in applying NMR spectroscopy to address fundamentally important biological questions relevant to the mission of the institution – to find cures for children with catastrophic diseases through research and treatment. The new position will be supported by generous startup funds and personnel. Candidates should have a Ph.D and/or MD degree, at least three years of relevant postgraduate experience, and a demonstrated track record of productivity.

Applicants should send a curriculum vitae, a 1-2 page summary of research interests and future plans, and the names of three references to: Dr. Stephen White, Endowed Chair, Department of Structural Biology, MS 311, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678.

St. Jude is an Equal Opportunity Employer and a Drug-Free Workplace.

Candidates receiving offers of employment will be subject to preemployment drug testing and background checks.

Federal law requires all employers to verify the identity and employment eligibility of all persons hired to work in the United States. To support this mandate, St. Jude Children's Research Hospital participates in E-Verify.

www.stjude.org



THE HONG KONG UNIVERSITY OF
SCIENCE AND TECHNOLOGY

Tenure-track Faculty Position(s) in Chemistry

The Department of Chemistry invites applications for two or three tenure-track faculty positions (preferably at the rank of Assistant Professor): one (potentially two) in the area of organic chemistry, and the other in the area of analytical chemistry.

Appointees are expected to teach analytical/organic chemistry at both undergraduate and postgraduate levels, and to develop a vigorous research program in their area of interest.

Applicants should have a PhD degree in chemistry or related fields and relevant postdoctoral experience. The starting salary will be commensurate with qualifications and experience. Fringe benefits including medical/dental benefits and annual leave will be provided. Housing will also be provided where applicable. Initial appointment will normally be on a three-year contract, renewable subject to mutual agreement. A gratuity will be payable upon successful completion of contract.

Applications including a curriculum vitae, a statement of teaching philosophy, a research proposal, and names and addresses of three referees (including email addresses) should be sent before **15 October 2009** to:

Chemistry Faculty Search Committee
Department of Chemistry
The Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, HONG KONG
Fax: (852) 3521-1486
Email: chemsearch@ust.hk

Additional information about the University and the Department can be found at <http://www.ust.hk>.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)



Neurogenetics Faculty Position The Department of Biology at The University of Iowa

Applications are invited to fill a tenure-track position at the Assistant Professor level with appointment in fall 2010. We are seeking candidates whose research uses genetic approaches to address basic mechanisms of neural development or function in model organisms. Successful candidates will have a strong background in research that complements the department's strength in neural and sensory system development and function, and teaching interests in genetics. The department has seen significant growth over the last five years, including establishment of the Carver Center for Genomics and the Carver Center for Imaging. The Department of Biology and the College of Liberal Arts and Sciences are strongly committed to gender and ethnic diversity; the strategic plans of the University, College and Department reflect this commitment. The Department of Biology is located in recently renovated space and provides competitive salaries and benefits along with strong infrastructure support for research. To obtain more information about the department and its research facilities, visit www.biology.uiowa.edu. Additionally, the department has a tradition of supporting the career development of its assistant professors.

Successful candidates must have a Ph.D. in one of the biological sciences or related areas, post-doctoral experience, a recognized record of accomplishment as reflected in publications, evidence of ability to establish and maintain an extramurally funded research program, and interest in participating in the department's teaching mission. Desirable qualifications include the potential for productive interactions with existing faculty, ability to introduce new experimental techniques or methodologies to the department, and recognition in the candidate's area of research.

Candidates should apply online under requisition 57039 at <http://jobs.uiowa.edu>. Application materials include a cover letter, curriculum vitae, a statement of research objectives and teaching interests, electronic copies of selected publications, and the names of at least 3 references. Formal screening of applications will begin **October 1, 2009** and continue until the position is filled. The University of Iowa ranks in the top 20 of public research universities (#13 in NIH funding) and is located in a culturally and ethnically diverse community.

The University of Iowa is an Affirmative Action/Equal Opportunity Employer. Women and minorities are especially encouraged to apply.



Faculty Position in Biochemistry

The Department of Biochemistry at The Ohio State University seeks to fill a **Tenure-Track Faculty Position**, preferably at the Assistant Professor level, as part of a multi-year effort to develop excellence in structure function research on biological macromolecules (see <http://www.biosci.ohio-state.edu/~biochem>). Candidates with research interests spanning all areas of biochemistry, including chemical and structural biology, nucleic acid-protein interactions, and membrane proteins, are encouraged to apply.

Potential candidates should have a Ph.D. degree in Biochemistry or a related discipline, at least two years of postdoctoral experience, and an established record of accomplishment in biochemical research. Additional interdisciplinary training will be viewed favorably. A commitment to the development of a vigorous and innovative independent research program supported with extramural funds, the integration of teaching and research, and the rigorous training and instruction of graduate and undergraduate students is essential.

Please send a curriculum vitae, a summary of research accomplishments, and a three-page description of future research plans to the **Faculty Search Committee, Department of Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210**. Candidates should also have three letters of recommendation sent to this address. For full consideration, applications should be received by **October 15, 2009**, but will be accepted until the position is filled.

In its commitment to build a diverse workforce, The Ohio State University encourages applications from minorities, veterans, women and individuals with disabilities. Flexible work options are available. OSU is an Equal Opportunity/Access and Affirmative Action Employer.

The Sanford Project:

A Major Multidisciplinary Research Initiative in Type 1 Diabetes

Sanford Research/USD seeks faculty and team leaders to direct and implement components of The Sanford Project, an emerging translational research center focused on type 1 diabetes (T1D). Concentrating on beta cell regeneration and autoimmunity, the Sanford Project (www.sanfordproject.org) is funded by Sanford Health, the \$400 million gift Sanford received from businessman Denny Sanford and a \$10 million donation from the Todd and Linda Broin family.

Sanford seeks qualified scientists of any rank to join its team. Successful candidates are expected to design and implement innovative T1D-related projects, build corresponding infrastructure and leverage the existing translational resources provided by Sanford Health. Candidates must prefer working in a team environment, identify and champion outside collaborations, obtain extramural funding and qualify for an academic appointment at the Sanford School of Medicine of The University of South Dakota. Candidates must have MD and/or PhD degrees. The level of appointment will be commensurate with experience and a comprehensive compensation, benefit and competitive start-up package will be provided.

Applications should include: detailed curriculum vitae; cover letter; past, current and future funding; experience and expertise; future plans; and the names and contact information of three references.

Application materials should be sent electronically (preferred) or by mail to:

Dr. Paul Burn
Todd & Linda Broin Chair
Director of The Sanford Project, Sanford Research/USD
Professor, Department of Pediatrics
Sanford School of Medicine of the University of South Dakota
900 West Delaware Street, Sioux Falls, SD 57104
Telephone: (605) 312-6020
burnp@sanfordhealth.org
www.sanfordhealth.org

Sanford Health is an Equal Opportunity/
Affirmative Action Employer.

The following positions are available, effective immediately:

Lead, Translational & Experimental Medicine, Regeneration Lead, Translational & Experimental Medicine, Autoimmunity

Successful candidates will identify, design and carry out experiments that are aimed at establishing "proof of concept" for clinical candidate molecules and novel approaches in humans for the T1D indication, will design and perform mechanistic studies in humans, and will develop and establish the corresponding experimental and translational capabilities, infrastructure and technologies.

Lead, Animal Models & in vivo Pharmacology, Regeneration Lead, Animal Models & in vivo Pharmacology, Autoimmunity

Successful candidates will identify, design and carry out experiments that are aimed at establishing "proof of concept" for clinical candidate molecules and novel approaches in the respective animal models of T1D, will design and perform supporting mechanistic and in vivo pharmacology studies, and will develop and establish the corresponding translational capabilities, infrastructure, technologies, and respective animal models.

Lead, Cellular Regeneration Biology Lead, Cellular Autoimmune & Inflammation Biology

Successful candidates will identify, design and carry out experiments in the respective cellular assay systems relevant for T1D, will design and perform supporting mechanistic and molecular studies, and will establish the corresponding capabilities, infrastructure, technologies and cellular systems.





United States Department of State Washington, DC

The United States Department of State invites applications for visiting scholar positions during the 2010-2011 academic years, per Section 202 of the Arms Control and Disarmament Act, as amended (22 U.S.C 2568).

Positions of **Physical Scientist, Biological Scientist, Chemical Scientist, and Political Scientist** are available at the Department of State in 2010-2011. The candidates selected under this program will have an opportunity for active participation in the arms control, nonproliferation, and disarmament activities of the Department of State and to enable the Department to gain the perspective and expertise such persons can offer.

The Department of State reimburses institutions of Foster Fellows for their salary and benefits, and Foster fellows receive travel reimbursement and a per diem allowance (subject to some restrictions).

For complete information about the program and positions, see <http://www.state.gov/t/>. To apply, please send a letter describing your perspective and expertise, a CV, three letters of reference, and two published articles to: **Thomas J. Yehl, VCI/TA, U.S. Dept. of State, Washington, DC 20520**. Receipt deadline is **September 30, 2009**.

Applicants must be U.S. citizens and will be subject to a security background investigation in the event of their selection for the program. Contact **Ms. Annette Day** at **202-647-4153** for more detailed information.



"The Future Grows Here"

SUPERVISORY RESEARCH ENTOMOLOGIST/CHEMIST/ OR PLANT PATHOLOGIST

Commodity Protection and Quality
Research Unit, Parlier, CA

**GS14/15; Salary Range of
\$95,010.00 to \$145,290.00**

The USDA, Agricultural Research Service (ARS) is seeking an outstanding research scientist for a permanent full-time Research Leader position. The incumbent will lead a multi-disciplinary team of scientists to develop effective postharvest commodity protection strategies to mitigate the effects of insect pests, including production pests when these subsequently become postharvest pests, on horticultural crops including fresh or dried fruit/vegetables and nuts. The incumbent will develop an individual research program in postharvest entomology, microbiology, plant physiology, chemistry or biochemistry that contributes to the research objectives of the Unit to maintain/enhance postharvest commodity quality, and developing new and improved postharvest pest control technology or methodology to meet domestic and foreign export market requirements. U.S. citizenship is required. A PhD is highly desirable. To apply, print a copy of vacancy announcement **ARS-X9W-0256** from the ARS Careers Website at <http://www.afm.ars.usda.gov/divisions/hrd/index.html> and follow the application directions provided. For questions about this position, call **Dr. Edwin Civerolo**, at **559-596-2702** or E-Mail: Edwin.civerolo@ars.usda.gov. Applications must be received by **September 18, 2009**. *USDA/ARS is an Equal Opportunity Employer and Provider.*



STOWERS INSTITUTE®
FOR MEDICAL RESEARCH

Faculty Search

The Stowers Institute seeks exceptional scientists at all levels to lead independent research programs using approaches in the areas of biochemistry, biophysics, cell biology, computational biology, development, genetics, genomics and neurosciences.

We offer substantial start-up packages and continuing intramural research support with access to core facilities that provide advice, training and service to enhance the Institute's interdisciplinary and collaborative research programs. Current core facilities are staffed by over 100 scientists with expertise in bioinformatics, cytometry, histology, imaging, microarray, next generation sequencing, transgenic and ES cell technologies, proteomics and molecular biology.

Candidates with Ph.D. and M.D. degrees, postdoctoral research experience and outstanding records of research accomplishment should send curriculum vitae and one peer-reviewed paper to investigator.search@stowers.org.

More information may be found at
www.stowers.org

The Stowers Institute is committed to equal opportunity in all its programs.

The University of Tokyo School of Science Department of Chemistry Professor of Organic Chemistry

for a tenure-track Full Professor or Associate Professor of Organic Chemistry. The successful applicant is expected to conduct advanced scientific researches independently and to contribute to the teaching of undergraduate and graduate students. Applicants should arrange to have two letters of recommendation (one from inside Japan and the other from outside Japan, directly sent to the Chemistry Department Chairman, hasegawa@chem.s.u-tokyo.ac.jp).

Applicants should also send a curriculum vitae, lists of publications, invited lectures, awards, and research funds, reprints of five original articles, a description of previous research activities and an outline of their future research plans (should be written in English) to the Chemistry Department Chairman, Prof. Tetsuya Hasegawa.

Email inquiries to:

- Prof. Kazuo Tachibana (ktachi@chem.s.u-tokyo.ac.jp)
- Prof. Eiichi Nakamura (nakamura@chem.s.u-tokyo.ac.jp)
- Prof. Shu Kobayashi (shu_kobayashi@chem.s.u-tokyo.ac.jp).

Deadline for applications is October 20, 2009.

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds.



The Friedrich-Alexander-Universität Erlangen-Nürnberg with its 26,000 students and 12,000 employees is one of the leading European research universities with a strong focus on engineering, natural sciences and medicine. The university is committed to excellent research and interdisciplinary academic education.

The research cluster of excellence „Engineering of Advanced Materials – Hierarchical Structure Formation for Functional Devices“ is supported by the German Excellence Initiative and focuses on the science and engineering of hierarchical materials organized from the molecular to the macroscopic levels and the related process technologies. Based on a coherent methodological approach the following research areas are explored:

- cross-cutting topics: particle technology, nanomaterial characterization and multiscale modeling and simulation
- engineering of nanoelectronic materials
- engineering of photonic and optical materials
- engineering of catalytic materials
- engineering of lightweight materials

The vision of the cluster is to bridge the gap between fundamental research and real-world applications of modern high-performance materials in key scientific and engineering areas.

As part of the cluster's **Rising Star Program** leadership positions are available for independent, interdisciplinary, and fundamental research. The Cluster invites applications for up to **two**

Junior Professorships (W1, tenure track)

Candidates with an excellent track record in at least one of the mentioned research areas are eligible to apply. As fellows of the program they will be granted flexible resources for three years and will be expected to establish a research group within the cluster. The successful candidate is expected to develop an internationally recognized fundamental research program, strongly interlinked with the cluster's research areas.

Applicants should be exceptional junior researchers with outstanding university undergraduate and doctoral degrees in natural sciences or engineering as well as excellent teaching skills. Applicants with post-doctoral employment in Germany should not exceed the maximum employment period of six years according to German law. Appointments are for three years initially with the possibility of a further extension for another three years, following a positive evaluation. Assuming success during this six year period and subject to the fulfillment of certain legal requirements, candidates will be offered promotion to the rank of a W2 Professor (tenure track).

The Universität Erlangen-Nürnberg actively encourages applications from female candidates in an effort to increase female representation in research and teaching. Given equal suitability for the appointment the applications of disabled candidates will be given priority.

The positions are available immediately.

Application documents (curriculum vitae, photograph, copies of degree certificates, and a selection of the most important publications) and a brief statement of research interests (not more than 3 pages) must be submitted before **September 25th, 2009** by e-mail to: Friedrich-Alexander-Universität Erlangen-Nürnberg, Cluster of Excellence Engineering of Advanced Materials, Prof. Dr.-Ing. Wolfgang Peukert, Nägelsbachstr. 49b, 91052 Erlangen, e-mail: administration@eam.uni-erlangen.de, www.eam.uni-erlangen.de

**Friedrich-Alexander-Universität
Erlangen-Nürnberg**



www.uni-erlangen.de



Worcester Polytechnic Institute

Dean of Arts and Sciences

Worcester Polytechnic Institute (WPI) invites nominations and applications for the position of Dean of Arts and Sciences. This new full-time academic administrative position will report to the Provost and Senior Vice President.

The Position: The founding Dean of Arts and Sciences will build on WPI's strengths in humanities, arts, sciences, social sciences, and mathematics. Under the dean's purview will be the departments of Biology and Biotechnology, Chemistry and Biochemistry, Computer Sciences, Humanities and Arts, Mathematical Sciences, Physics, and Social Science and Policy Studies. WPI is committed to the highest quality scholarship and teaching in these areas. The dean will help solidify WPI's leadership in a number of research areas, including a major strategic commitment to the life sciences. The dean will also enhance the external visibility of WPI's distinctive educational programs, and continue its tradition of innovative research and education, including interdisciplinary programs within and beyond the arts and sciences.

The University: Founded in 1865, WPI is one of the nation's oldest technological universities. Today, WPI is a highly selective private university with over 3,900 undergraduate and graduate students enrolled in more than 50 programs in science, engineering, technology, management, the social sciences, and the humanities and arts. WPI is consistently ranked among the top national universities in U.S. News & World Report. Its innovative project-enriched curriculum engages students and faculty in real-world problem solving, often at one of WPI's 20 project centers in North America and Central America, Africa, Australia, Asia, and Europe. The university is located in Worcester in the heart of Massachusetts and an hour away from Boston.

Qualifications: The dean must be devoted to excellence in research and in undergraduate and graduate education; committed to successful collaboration and cooperation within the university; and willing to invest substantial amounts of time in external relations, including fundraising and building connections with alumni, foundations, corporations, government agencies, and cultural institutions. The successful candidate must possess an earned doctorate in a relevant field and have a distinguished record of academic achievement in both teaching and research. Substantial experience in administrative leadership and management in academic positions of significant responsibility, including personnel and budgetary management, resource allocation, and accreditation processes, is also necessary. Candidates must have a sustained track record of personal success in program development and obtaining significant external funds, as well as strong interpersonal skills.



Information for Applicants: The review of credentials will begin immediately and will continue until the position is filled. For best consideration, please submit materials before October 21, 2009. Application materials should include: a letter describing your interest in and qualifications for the position; a curriculum vitae; and the names, addresses (including email), and telephone numbers of at least five references which may include trustees, administrators, faculty, students, and community leaders. *Correspondents are strongly encouraged to communicate by email utilizing Adobe Acrobat or Word attachments.*

All nominations and applications shall be confidential. Direct requests for information and written nominations and applications to:

Steve Leo, Principal • Vicki Henderson, Senior Associate
Storbeck/Pimentel & Associates, LLC • Rose Tree Corporate Center II
1400 North Providence Road, Suite 6000 • Media, PA 19063
s.leo@storbeckpimentel.com • v.henderson@storbeckpimentel.com
610-572-4296

To enrich education through diversity, WPI is an affirmative action, equal opportunity employer.

POSITIONS OPEN

C · H · O · R · I

Children's Hospital Oakland Research Institute

STEM CELL OR TRANSPLANTATION BIOLOGY RESEARCH SCIENTIST

The Blood and Marrow Transplantation Program (BMT) at Children's Hospital & Research Center, Oakland and Children's Hospital Oakland Research Institute (CHORI) seek a full-time, mid-level faculty member to conduct basic and/or translational research related to stem cell or hematopoietic cell transplantation biology.

The optimal candidate will have demonstrated successful experience in translational and/or basic research in stem cell or transplantation biology and peer-reviewed independent funding. We seek a physician scientist or laboratory scientist who would complement existing research efforts in stem cell biology and transplantation for red blood cell disorders.

CHORI, the research branch of Children's Hospital & Research Center Oakland, ranks sixth in NIH funding among U.S. children's hospitals ([website: http://www.chori.org](http://www.chori.org)). The BMT Program is a Foundation for the Accreditation of Cellular Therapy-accredited pediatric clinical transplant program with a focus on translational research in hemoglobin disorders. This new position is supported in part by a large gift to the BMT program to expand and broaden its existing research program.

Interested applicants should submit their curriculum vitae to:

Mark Walters, M.D.

Director, Blood and Marrow Transplant Program
Children's Hospital & Research Center, Oakland

747 52nd Street

Oakland, CA 94609

Telephone: 510-428-3374

E-mail: mwalters@mail.cho.org

Children's Hospital & Research Center, Oakland is an Equal Opportunity Employer. Applications from qualified women and underrepresented minorities are particularly encouraged.

THIS IS A GRANT-FUNDED POSITION

The Department of Physiology is seeking an **ASSISTANT (ASSOCIATE) RESEARCH SCIENTIST** to assist in developing projects in the field of cardiac cell biology and biochemistry. The projects specifically focus on understanding the role of inflammation on cardiac repair and stem cell biology. Applicants should have an M.D., Ph.D., D.V.M., or equivalent degree with a strong background in small animal surgery. Familiarity with human physiology, echocardiography, and hemodynamics is preferred. We are offering an interesting job opportunity in an innovative surrounding and with very attractive salary. Interested applicants should send a letter stating their background together with curriculum vitae to:

Abdelkarim Sabri, Ph.D.

Associate Professor

E-mail: sabri@temple.edu

Temple University is an Affirmative Action, Equal Opportunity Employer that is interested in the recruitment of diverse faculty and staff.

FACULTY POSITION

Institute of Molecular and Cellular Biology
National Taiwan University

The Institute is seeking an outstanding individual to fill a full-time Faculty position available on August 1, 2010. The level of appointment is open. The specific research area should be related to molecular biology and/or cellular biology of plant or animal. Candidate must have a Ph.D. degree, and a postdoctoral experience is preferred. Applicant should submit curriculum vitae, a brief statement of research and teaching course(s), and three recommendation letters prior to November 30, 2009, to: Chair, Faculty Search Committee, Institute of Molecular and Cellular Biology, National Taiwan University, No.1, Section 4, Roosevelt Road, Taipei, Taiwan 10617. Website: <http://cell.lifescience.ntu.edu.tw/english/index.htm>.

POSITIONS OPEN

ASSISTANT PROFESSOR, Plant Biochemistry Position Announcement

The Department of Horticulture and Crop Science at The Ohio State University (OSU), in conjunction with the Plant Molecular Biology and Biotechnology (PMBB) Program, invites applications for a **PLANT BIOCHEMIST**. This is a tenure-track, Assistant Professor-level, nine-month, 90:10 research:teaching appointment. The Plant Biochemist will: (1) develop an interdisciplinary, extramurally funded program focused on fundamental processes related to bioenergy or bioproducts; (2) conduct research that increases our understanding of the biochemistry underlying plant-based compounds; and (3) develop a course that focuses on the applicant's area of expertise.

This position is one of several comprising the Translational Plant Sciences emphasis within the PMBB program funded by the Targeted Investment in Excellence initiative. Applicants who integrate modern approaches from biochemistry and chemistry with genomics, metabolomics, molecular biology, and biotechnology are preferred. Applicants whose research interests will enhance fuel, food, human health, or environmental quality are particularly encouraged to apply.

Based at the OSU Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio, the successful candidate will have the opportunity to collaborate with faculty in multidisciplinary programs, centers, and academic departments of OSU and nearby institutions. Extensive interaction with the future eminent scholar hire in plant biochemistry is anticipated. A newly renovated laboratory space and competitive startup funds are available.

A Ph.D. in biochemistry, plant physiology, chemistry, or other relevant discipline is required. Strong research experience including postdoctoral experience in a contemporary area of the plant sciences, an excellent publication record, and grantsmanship are expected.

Additional details on the position are available at [website: http://hcs.osu.edu](http://hcs.osu.edu). The search committee will start reviewing applicants on November 1, 2009, and will continue until a suitable applicant is found. Please submit curriculum vitae, a maximum two-page statement of research plans, and a maximum one-page description of teaching experience and goals to: **Dr. John Finer, Search Committee Chair, Department of Horticulture and Crop Science, The Ohio State University, OARDC, 1680 Madison Avenue, Wooster, OH 44691-4096**, preferably electronically to e-mail: finer.1@osu.edu. Candidates should also arrange to have three reference letters sent directly via e-mail to the Search Committee Chair.

To build a diverse work force, Ohio State encourages applications from individuals with disabilities, minorities, veterans, and women. Equal Employment Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR The University of Chicago, Department of Chemistry

The Department of Chemistry of the University of Chicago invites applications from outstanding individuals for the position of Assistant Professor of chemistry. This search is in the areas broadly defined as inorganic, organic, and physical chemistry. Applicants must apply online at the University of Chicago academic job [website: https://academiccareers.uchicago.edu](https://academiccareers.uchicago.edu). Applicants must upload a cover letter, curriculum vitae with a list of publications, and a succinct outline of research plans. The cover letter should be addressed to the Inorganic Search Committee, Organic Search Committee, or Physical Search Committee, depending on the applicant's discipline of interest. Applicants must also arrange to have three letters of recommendation sent to: **Department of Chemistry, Office of the Chairman (SCL 119), The University of Chicago, 5735 S. Ellis Avenue, Chicago, IL 60637**. The recommendation letters will be accepted by mail only. Review of completed applications will begin October 1, 2009; to ensure full consideration, all material should be submitted by that date. *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

UCLA

SYSTEMS BIOLOGY AND PHYSIOLOGY

The UCLA Department of Physiological Science invites applications for a tenure-track faculty position at the level of **ASSISTANT PROFESSOR**, beginning as early as July 2010. Exceptional candidates may be considered at a high rank. We seek applicants from any area of physiology, although we have particular interest in systems biology and understanding emergent properties of complex physiological systems (see [website: http://www.physci.ucla.edu](http://www.physci.ucla.edu) for more information and to apply). The successful candidate will be expected to participate in undergraduate and graduate teaching and to establish a vigorous, externally funded research program. Review of applications will begin October 5, 2009, but applications received afterward will be considered until the position is filled. Please use the following **job number: 0845-0708-01** in all correspondence. Salary is commensurate with experience. *The Department has a strong commitment to the achievement of excellence and diversity among its faculty, staff, and students. UCLA is an Equal Opportunity/Affirmative Action Employer.*

CELL BIOLOGIST

The Department of Biology at the College of the Holy Cross seeks a tenure-track Cell Biologist to begin August 2010. The appointee will teach an intermediate laboratory course in cell biology and an upper-division course compatible with current offerings, and participate in introductory teaching for biology majors, pre-health students, and nonscience majors. Candidates must demonstrate commitment to, and excellence in, undergraduate teaching as well as scholarly achievement, and propose a research program involving undergraduates.

This position carries a three/two teaching load with a full-salary, one-semester research leave before tenure review and generous sabbatical and fellowship leaves for senior faculty. Ph.D. required at time of appointment. For further information, see [website: http://www.holycross.edu/departments/biology/website](http://www.holycross.edu/departments/biology/website).

Applicants should submit a cover letter, statements of research interests and teaching philosophy, curriculum vitae, all academic transcripts, publications, and three letters of recommendation in hard copy by 15 October 2009 to: **Dr. Mary Lee Ledbetter, Ref: SCI, Search Committee, Department of Biology, College of the Holy Cross, Worcester, MA 01610**. Holy Cross is a highly selective Catholic liberal arts college in the Jesuit tradition (enrollment 2,700) located in a medium-sized city 45 miles west of Boston. It belongs to the Colleges of Worcester Consortium ([website: http://www.cowc.org](http://www.cowc.org)) and the New England Higher Education Recruitment Consortium ([website: http://www.faculty.harvard.edu/01/013.html](http://www.faculty.harvard.edu/01/013.html)).

The College is an Equal Employment Opportunity Employer and complies with all federal and Massachusetts laws concerning equal opportunity and affirmative action in the workplace.

POSTDOCTORAL POSITION, ANTIMICROBIAL DRUG DISCOVERY. Position available immediately to investigate novel approaches to antimicrobial discovery targeting bacterial pathogens. Experience in molecular biology and protein biochemistry required, and protein X-ray crystallography or enzymology desired. Submit curriculum vitae, statement of research interests, and names of three references to: **Dr. M. Ashley Spies and Dr. Steven Blanke, University of Illinois, Urbana, IL** at e-mail: aspies@illinois.edu and sblank@illinois.edu.

POSTDOCTORAL FELLOW POSITION

A Postdoctoral position for three years is available to study signaling pathways that regulate cancer stem cells in brain tumor. Experience in molecular biology and mouse model is preferred. Applicant should have Ph.D. or M.D. and send curriculum vitae and three names of references to: **Dr. Xing Fan, e-mail: xingf@umich.edu; Department of Neurosurgery and Cell and Development Biology, University of Michigan, Ann Arbor, MI.**

A nondiscriminatory, Affirmative Action Employer.



Announcement for the Search for the President of Okinawa Institute of Science and Technology

Background: The Okinawa Institute of Science and Technology (OIST: <http://www.oist.jp>) is a new international research university of science and technology in Okinawa, Japan. The aim of OIST is to build a graduate university of international standing for the promotion of science and technology, create a true center of excellence in the Asia-Pacific region and contribute to the sustainable development of Okinawa. The university will be committed to achieving global networking and collaboration with industry. Under the leadership of the founding President Sydney Brenner and the OIST Board of Governors, the first stage of construction of the permanent campus will be complete in early 2010. Twenty-one research units (11 international, 10 Japanese) have already been established, with over 160 researchers (50 international) in temporary laboratories. Graduate students will be accepted in 2010 through joint agreements with other universities. The next stage is the development of the campus for about 50 faculty members and accreditation as an independent university prior to opening in 2012. The initial areas of research are neuroscience, molecular science, mathematical and computational biology, and environmental science, but the aim is to promote research which is highly interdisciplinary, adding and integrating such core areas as bioscience, chemistry, physics, computer and information science, mathematics, and engineering. With the mission to promote international research, the language of instruction is English and around half of the faculty, researchers and graduate students will be foreign nationals. Initial funding will be provided by the Japanese government, but independent funding is expected to increase steadily over time. The campus is located on 85 hectares of undeveloped semitropical forest overlooking beautiful beaches and reefs in Onna-son on the west coast of Okinawa, with adequate provision for housing and international schooling.

Duties and responsibilities: (1) The position as President will be a 5-year full-time and renewable appointment. (2) The President is appointed by the Board of Governors of OIST. The Board has final authority for the finances and appointments of the university. Close cooperation between the President and the Board is essential to the success of the university. (3) The term of the President begins with the establishment of the university in late 2011 or early 2012. The President-elect will be appointed as an establishment committee member until then and will work with the Board to ensure the opening of the new OIST campus facility in 2012, provide directions for the expansion of the university, be actively involved in the recruitment of scientists of international standing, and in the building of a first rate international university. (4) The President has full responsibility for the operations of the Okinawa Institute of Science and Technology and should work closely with the other officers and senior management of the university. The responsibilities involve the hiring of outstanding faculty and researchers, developing strategic plans for the university and overseeing research, academic affairs and budget. Additional responsibilities are the building of academic and industrial partnerships, and the fostering of positive relations with and encouraging the development of the Okinawa community. (5) International recruitment and fund-raising will require that the President travel periodically. However, even during periods of absence, the President should maintain close involvement in the operations of OIST.

Qualifications: The President of OIST must have the following qualifications: (1) Excellent scientific credentials to enable recruitment of first rate faculty and research scientists and the formation of partnerships with other universities and institutions; (2) Experience in university administration and teaching to be able to utilize effectively and efficiently the resources available to the University; (3) Experience of working in an international environment; (4) Excellent communication skills to ensure good public relations with scientists, government, and the community; (5) There is no restriction with regard to age or nationality.

Applications: Applications should include: (1) a cover letter addressed to OIST President Search Committee; (2) CV; (3) statement of interest and relevant research and administrative accomplishments and (4) names and contact information for 5 references. Submit applications electronically to oistpresidentsearch@oist.jp or by post mail to **Attn: OIST President Search, 7542 Onna, Onna-son, Okinawa, Japan 904-0411**. Applications will be reviewed as they are received, and the position will remain open until it is filled.

EVMS

Eastern Virginia Medical School

Chair DEPARTMENT OF PHYSIOLOGICAL SCIENCES

Eastern Virginia Medical School (EVMS) is seeking an extraordinary individual to Chair its Department of Physiological Sciences. EVMS has made a major commitment to strengthen, expand, and integrate the academic enterprise in basic and clinical research and to develop an academic culture which places EVMS at the forefront in biomedical science, education, and health delivery. The Hampton Roads - Mid Atlantic region offers exceptional education, recreation, entertainment and housing opportunities. The new Chair of Physiological Sciences will have exceptional support for recruiting new faculty and building nationally prominent research and education programs. Candidates for this position must have a PhD and/or MD degree, a distinguished record of scholarly achievements including international recognition in research, an outstanding record of maintaining extramural research funding, and demonstrated commitment to excellence in teaching. Candidates should have superb leadership and interpersonal skills and the ability to develop programmatic research areas that bridge the Department's three divisions (biochemistry, pharmacology and physiology). Major current research focal areas are diabetes/metabolic diseases, reproductive biology and cardiovascular science with funding from the National Institutes of Health and private foundations. Candidates with research expertise and NIH R01-type grant support in these areas are especially encouraged to apply. Faculty provide instruction to students in the first and second year of medical school, in the allied health professions, and train M.S. and Ph.D. graduate students in the biomedical sciences. The successful candidate will be expected to develop program project grants, articulate and implement a vision for development of the department and recruit talented research scientists in ways that enhance current research and educational programs, and that may include strong programs in new areas of cutting edge research. For further information, please visit the Department Web site: <http://www.evms.edu>.

All inquiries, nominations, and applications will be held in strictest confidence. Applicants should forward electronically (hubandsb@evms.edu) a letter of interest along with (1) curriculum vitae, (2) NIH biosketch and (3) research interests to: **Chair, Department of Physiological Sciences Search Committee, Eastern Virginia Medical School, Post Office Box 1980, Norfolk, VA 23501-1980.**

EVMS is an Equal Opportunity/Affirmative Action and Drug Free Workplace Employer and encourages applications of women and minorities.



Faculty Positions in Cell Signaling at the University of Houston

The DEPARTMENT OF BIOLOGY AND BIOCHEMISTRY AT THE UNIVERSITY OF HOUSTON announces formation of the new Center for Nuclear Receptors and Cell Signaling under the direction of Professor Jan-Åke Gustafsson. Recruiting into this center will occur over a period of three years, and it will ultimately be comprised of twelve tenured and tenure-track faculty with distinct but complementary research interests surrounding nuclear receptors and cell signaling. The scientific environment at the University of Houston is excellent, especially in areas such as neurobiology, structural biology, computational life sciences, and bioinformatics, and we envision that this new center will develop multiple areas of basic life sciences as well as its applications. In addition, the Center is geographically located in new research space close to numerous academic institutions and hospitals in the Texas Medical Center.

We now invite applications for tenured or tenure-track faculty at all levels in the areas of the biology of nuclear receptors, including neurobiology, cancer biology, developmental biology, immunology and cell biology. The successful applicants will complement existing departmental strengths in neuroscience, developmental biology, cell and molecular biology and mechanisms of transcriptional regulation. We are especially interested in recruiting middle level and senior level faculty with active externally funded research programs. The positions require a Ph.D. and postdoctoral experience in appropriate areas of life sciences. Faculty in the center are expected to develop and/or maintain nationally competitive externally funded research programs and to participate in graduate and undergraduate teaching. The Department and the Center have spacious new laboratories and well-equipped core facilities and encourage research collaborations.

Submit curriculum vitae, a research plan, and the names of three references sent electronically as a single file to: cellsignaling@uh.edu. Applications will be reviewed as they are received.

UH is an Equal Opportunity/Affirmative Action Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.

POSITIONS OPEN

MOLECULAR NEUROBIOLOGIST

The Department of Biology and the Neuroscience Program at Amherst College invite applications for a tenure-track position at the **ASSISTANT PROFESSOR** level in molecular neurobiology. The research program of the successful candidate will be one that can involve undergraduate biology and neuroscience majors. Teaching duties include development of a laboratory course in molecular neurobiology, teaching other courses in the candidate's area, as well as rotation in team-taught introductory biology and neuroscience courses. A completed Ph.D. is required and postdoctoral experience is expected. Send curriculum vitae and statements of research and teaching interests to: **Neurobiology Search Committee, Department of Biology, Amherst College, Amherst, MA 01002-5000**. Electronic submissions will not be accepted. Have three letters of recommendation sent separately.

Review of applications will begin October 23, 2009, and continue until the position has been filled.

Amherst is a private, undergraduate liberal arts college for men and women, with 1,650 students and a teaching faculty of 200. Located in the Connecticut River Valley of western Massachusetts, Amherst participates with Hampshire, Mount Holyoke, and Smith Colleges and the University of Massachusetts in the Five College Consortium.

Amherst College is an Equal Opportunity Employer and encourages women, persons of color, and persons with disabilities to apply. The College is committed to enriching the diversity of its faculty, administration, and staff to ensure that full participation and inclusion are an integral part of the culture of the institution.

FACULTY POSITION

Columbia University Medical Center Department of Genetics and Development

The Department of Genetics and Development at Columbia University Medical Center is expanding its faculty and seeks outstanding applicants with the ability to develop an independent research program for a tenure-track position at the **ASSISTANT PROFESSOR** level. In special circumstances, applicants at other levels will also be considered. Applicant's research program should use molecular genetic approaches to study, in any model organism, questions relevant to vertebrate physiology and to the molecular bases of human degenerative diseases. Our Department spans a broad range of interests including developmental biology, physiology, DNA repair and recombination, cancer, and human genetics. Applicants should include curriculum vitae and a summary of current and proposed research programs and should arrange for three letters of reference to be sent.

Completed applications should be submitted via website: <https://academicjobs.columbia.edu/applicants/Central?quickFind=51990>.

Consideration of completed applications will begin September 30, 2009.

Columbia University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

UNIVERSITY OF ROCHESTER. The Department of Chemistry invites applications in all areas of chemistry at the **ASSISTANT, ASSOCIATE, and FULL PROFESSOR** levels. Candidates are expected to establish an outstanding program of original research and to be effective teachers at the graduate and undergraduate levels. Application materials should preferably be submitted online at website: <https://www2.chem.rochester.edu/facultyapp/>. Alternatively, send curriculum vitae indicating graduate and postdoctoral advisers along with a statement of research plans and teaching interests and arrange for three letters of recommendation to be sent to: **Ms. Karen Dean** in electronic form (preferred) at e-mail: dean@chem.rochester.edu, or by mail to: **Chemistry Faculty Search Committee, c/o Ms. Karen Dean, Department of Chemistry, University of Rochester, RC Box 270216, Rochester, NY 14627-0216**. Review of completed applications will begin on October 1, 2009. *The University of Rochester has a strong commitment to diversity and actively encourages applications from candidates from groups underrepresented in higher education. The University is an Equal Opportunity Employer.*

POSITIONS OPEN

CELL BIOLOGIST AND GENETICIST. The Biology Department of Franklin & Marshall College (website: <http://www.fandm.edu/biology>) invites applications for two tenure-track **ASSISTANT PROFESSOR** positions, one in cell biology and one in genetics, beginning fall 2010. Candidates should have the Ph.D. and demonstrated strengths in teaching and research.

The teaching load is three/two, and responsibilities for each position include: (1) lecture and laboratory sections of team-taught courses in sophomore-level cell biology or upper-level genetics (population to molecular); (2) an advanced laboratory elective in the candidate's area of expertise; and (3) participation in the College's general education curriculum. Successful candidates will have opportunities to participate in interdisciplinary programs, including biochemistry and molecular biology, bioinformatics, animal behavior, neuroscience, and public health.

F&M has a tradition of excellence in faculty/student collaborative research in the sciences. In 2007, the Biology Department moved into a 100,000 square foot state-of-the-art interdisciplinary teaching and research facility. In 2008, F&M received a Howard Hughes Medical Institute award to launch a bioinformatics program.

Applicants should arrange to have letters sent from three references, and should submit a letter of application, curriculum vitae, copies of graduate and undergraduate transcripts, teaching evaluations, and a statement that explains plans for actively engaging undergraduates through teaching and research and that describes goals for developing as a teacher and scholar. Electronic applications will not be accepted. Priority will be given to complete applications received by October 9, 2009. Applications should be sent to the search committee chair (**Dr. Carl Pike** for cell biology; **Dr. Clara Moore** for genetics) at: **Department of Biology, Franklin & Marshall College, P.O. Box 3003, Lancaster, PA 17604**. Telephone: 717-291-4118; fax: 717-358-4548; e-mail: janice.kaufman@fandm.edu.

Franklin & Marshall College is a highly selective liberal arts college with a demonstrated commitment to cultural pluralism. Equal Opportunity Employer.

UNIVERSITY OF OKLAHOMA BIOFUELS INITIATIVE

The University of Oklahoma is expanding a multi-department biofuels initiative, supported by the National Science Foundation Experimental Program to Stimulate Competitive Research and the Oklahoma State Regents for Higher Education, to strengthen and complement existing interdisciplinary programs in science and engineering fields, including botany and microbiology, chemical engineering, chemistry and biochemistry, and mechanical engineering.

As part of this initiative the University invites applications for tenure-track **ASSISTANT PROFESSOR** positions. We are seeking two individuals to develop world-class research programs related to biofuels generation and/or utilization. The desired areas of expertise are, for example (but not limited to): algae production or processing, bacterial engineering, bioinformatics, carbohydrate chemistry/biochemistry, chemical/biochemical characterization, combustion, pyrolysis, plant genetic engineering, and separation processes.

Candidates must have a Ph.D. or equivalent terminal degree and a record of research accomplishment. The successful individuals will also be expected to contribute to undergraduate and graduate education and provide leadership for the Biofuels Initiative. Applicants should submit curriculum vitae, a description of their research plans, and a brief statement of their teaching interests and philosophy. Applicants should also request three letters of reference be sent to the Search Committee. Application materials should be sent in electronic format (PDF or Word) to e-mail: biofuels.faculty.search@ou.edu.

Review of applications will begin on October 15, 2009, and continue until both positions are filled. Minorities and women are especially encouraged to apply. *The University of Oklahoma is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

POSTDOCTORAL POSITION available immediately for NIH-funded project to study cellular microbiology of tick-pathogen-vertebrate interactions. Training in protein-protein interactions and microscopy are desired. Please send curriculum vitae and three references to: **Dr. R. W. Stich, University of Missouri, Department of Veterinary Pathobiology, 210 Connaway Hall, Columbia, MO 65211**. E-mail: stichrw@missouri.edu.

POSTDOCTORAL POSITION, Cancer Research Sunnybrook Health Sciences Centre, University of Toronto

A Postdoctoral position is available in the laboratory of **Jorge Filmus**. The position requires a Ph.D. in biochemistry, molecular biology, or cell biology. Experience with mouse models, tissue culture, microscopy, and genetic and protein techniques is desirable. The laboratory is located within the discipline of molecular and cellular biology at the Sunnybrook Research Institute with access to all of the opportunities provided by the strong research environment in Toronto.

Interested candidates should send curriculum vitae, letter of interest, and names of three references to e-mail: jorge.filmus@sri.utoronto.ca. We express our appreciation to all applicants. However, only those to be interviewed will be contacted.

In accordance with the University of Toronto's employment equity policy and Sunnybrook Health Sciences Centre's diversity initiative, we encourage applications from qualified women and men, members of visible minorities, aboriginal peoples, and persons with disabilities.

CAREER OPPORTUNITY

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Contact the **Admissions Office**, telephone: 800-824-5526 at the New England College of Optometry, 424 Beacon Street, Boston, MA 02115. Additional information at website: <http://www.neco.edu>; e-mail: admissions@neco.edu.

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